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Marine Fungi of U.S. Gulf of Mexico Barrier Island Beaches: Biodiversity and Sampling Strategy

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MARINE FUNGI OF U.S. GULF OF MEXICO BARRIER ISLAND BEACHES: BIODIVERSITY AND SAMPLING STRATEGY

by

Allison Kathleen Walker

Abstract of a Dissertation
Submitted to the Graduate School
of The University of Southern Mississippi
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ABSTRACT

MARINE FUNGI OF U.S. GULF OF MEXICO BARRIER ISLAND BEACHES: BIODIVERSITY AND SAMPLING STRATEGY

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Marine fungi are an important but often overlooked component of marine ecosystems. Primarily saprotrophic, they are vital to coastal nutrient cycling processes and food webs. However, basic marine fungal distribution data are lacking in many parts of the world, as is knowledge of the sampling intensity required to characterize the biodiversity of these communities. The roles of substrate, season and latitude in shaping intertidal ascomycete community structure were examined for the U.S. Gulf of Mexico, and the role of sampling frequency on species richness estimates was also addressed. Best sampling practices were developed and 750 collections of beach detritus, sand and seafoam were made from the Florida Keys north to St. Vincent Island, Florida, from South Padre Island north to Galveston Island, Texas and from West Ship Island, Mississippi. Intertidal beach substrates were collected in winter and summer 2008-2009 from three Texas and four Florida barrier islands and incubated in the laboratory for six to twelve months to study fungal succession. Sampling was conducted every other month at West Ship Island, Mississippi from April 2009 through February 2010 and weekly at East Beach, MS during May 2010 to investigate changes in marine fungal communities over shorter timescales. Morphological and molecular techniques (ITS T-RFLP community fingerprinting, ITS gene sequencing) were employed to characterize and compare intertidal ascomycete communities. Species occurrence and abundance data

were used to determine biogeographical patterns of marine fungal distribution on abundant intertidal substrates.

Diversity indices and results from MDS and ANOSIM analysis of T-RFLP data indicate marine ascomycete diversity may increase with decreasing latitude. Substrate type strongly influenced fungal community structure. Most ascomycete species were substrate-specific, but several were found U.S. Gulf-wide on a variety of intertidal substrates. Substrates rich in lignocellulose (wood, saltmarsh plant detritus) housed the greatest ascomycete diversity, and seafoam provided a conservative snapshot of the intertidal fungal community as a whole. Sand was species-poor and dominated by *Corollospora maritima*, the most frequently-occurring marine fungus encountered in this study, indicating most marine fungi may arrive with their substrates and not become resident in sand. A seasonal trend was noted U.S. Gulf-wide for marine plant detritus only, which showed higher ascomycete diversity in winter. This study has increased the number of fungal species reported from the Gulf of Mexico by over 60%. Texas and Florida sampling was completed prior to the Deepwater Horizon oil spill (20 April 2010), providing valuable baseline data for the U.S. Gulf of Mexico. The diversity of species and substrates encountered over four weeks of sampling on East Beach, MS and ten months of sampling on West Ship Island, MS indicates more intensive sampling is required to fully characterize marine fungal communities of the highly dynamic intertidal zone of the GOM.

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DEDICATION

I dedicate this work to my husband Jake Walker, our new son Henry Walker and our families for constant inspiration and support in our shared appreciation of and curiosity about the natural world.

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LIST OF ABBREVIATIONS

ANOSIM	analysis of similarity
ASWA	antibiotic saltwater agar
bp	base pair, represents a unit of length or the position in a double stranded nucleotide sequence.
DNA	deoxyribonucleic acid
dNTP	deoxyribonucleotide triphosphate
DO	dissolved oxygen
FAM	6-carboxyfluorescein
GOM	Gulf of Mexico
ITS	internal transcribed spacer region. A highly variable, non-coding, multi-copy gene region in ribosomal DNA frequently used in fungal molecular systematics
L	liter
M	molar
MDS	non-metric multidimensional scaling
mg	milligram
mL	milliliter
mM	millimolar
NCBI	National Center for Biotechnology Information
ng	nanogram
PCR	polymerase chain reaction
PDA	potato dextrose agar

pmol	picomole
rDNA	ribosomal deoxyribonucleic acid
rpm	revolutions per minute
TBE	Tris-Borate-EDTA electrophoresis buffer
T-RFLP	Terminal Restriction Fragment Length Polymorphism
μL	microliter
μM	micromolar
UV	ultraviolet radiation
V	voltage

CHAPTER I

REVIEW OF FUNGI IN MARINE ENVIRONMENTS AND JUSTIFICATION FOR FURTHER RESEARCH

Introduction

Fungi in marine environments influence ecosystem and human health in a variety of ways, many of which are only beginning to be examined. Negative economic impacts stem from pathogenesis of recreational, commercial and captive-raised fish and crustaceans, corals, seagrass beds and salt marshes, all of which can translate into loss of fisheries nursery habitat and possibly human disease. Industrial, bioremedial and pharmaceutical applications point to ways in which marine fungi can enhance human quality of life, as well as their potential role as indicators of ecosystem health in the above-mentioned industries and habitats. Finally, studies indicate that marine fungi play a significant degradative and biochemical role in marine and estuarine environments, and additionally serve as an important food resource for invertebrates.

Definition

The existence of a unique marine mycota has been discovered mostly within the past 50 years. Marine fungi are distinct from terrestrial and freshwater fungi, both in their morphology and their adaptations to an aquatic habitat (Barghoorn and Linder 1944, Johnston and Sparrow 1961, Jones 1976, Kohlmeyer and Kohlmeyer 1979, Meyers 1996, Jones 2000). Additionally, marine fungi are an ecological, not taxonomic, group and cannot be defined by nutritional or physiological requirements (Kohlmeyer et al. 2004). Kohlmeyer and Kohlmeyer (1979) proposed the terms obligate marine fungi and facultative marine fungi. Obligate marine fungi are those that grow and sporulate

exclusively in a marine or estuarine habitat and are permanently or intermittently submerged. Facultative marine fungi are those that normally occupy freshwater or terrestrial habitats but are able to grow (and possibly to sporulate) in the marine environment. Marine fungi are microscopic; the largest marine ascomycetes and basidiomycetes are only 4-5 mm in diameter (Kohlmeyer et al. 2004). In general, they are less well-studied than terrestrial fungi and their ecological roles are not well known. While this review will focus on marine filamentous ascomycetes, other fungi such as ascomycete and basidiomycete yeasts, filamentous basidiomycetes, zygomycetes and chytrids have been reported from marine environments and require further study. The most recent phylogeny of major fungal lineages is given in Figure 1.

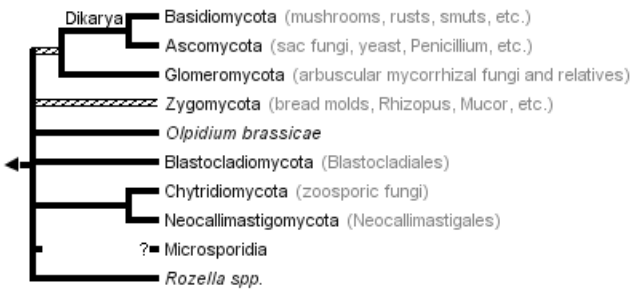

Figure 1. Current five-gene phylogeny of the major fungal lineages (modified from Blackwell et al. 2012).

History

The first obligately marine fungal species was discovered on rhizomes of the seagrass *Posidonia oceanica* (Durieu and Montagne 1869). Other early references to marine fungi were made by Desmazières (1849), and Crouan and Crouan (1867). In 1909 there were only 16 known species of marine fungi (Cotton 1909). Barghoorn and Linder (1944) described ten new genera and 25 species of lignicolous marine fungi, but it was not until the first monograph, containing 100 species, was published (Johnson and Sparrow 1961) that any substantial interest in marine fungi was generated (Figure 2).

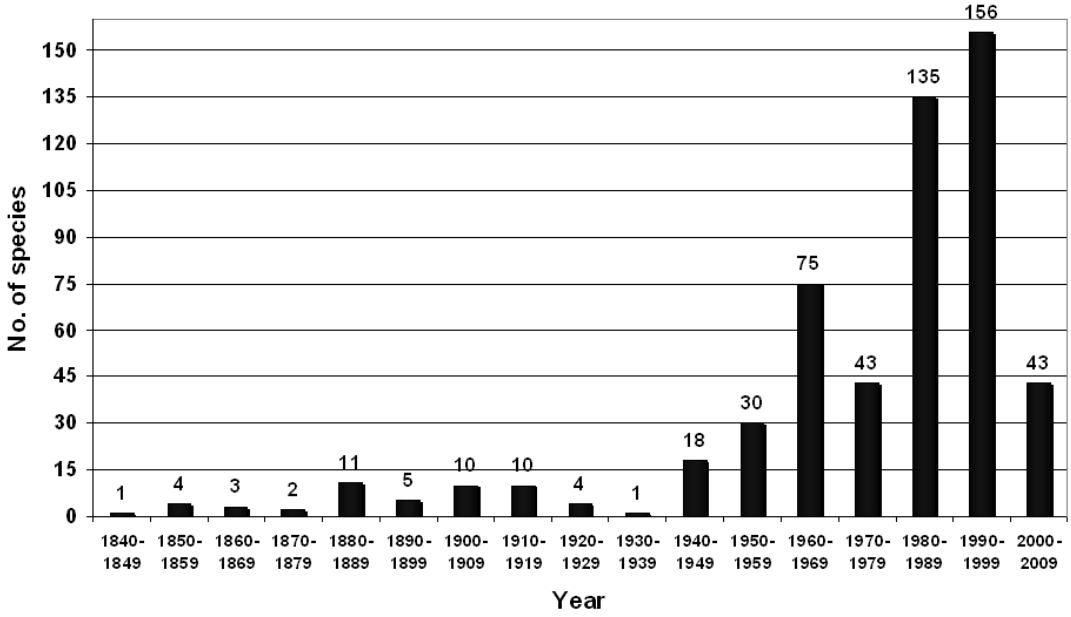

Figure 2. Number of new species of marine fungi described per decade from1840-2009 (Jones et al. 2009).

Almost 90% of all recognized higher marine fungi have only been described in the last 50 years. As of the most recent monograph, there are currently 530 described species of higher filamentous fungi that are recognized as obligate or facultative marine fungi (Jones et al. 2009). The majority of these are ascomycetes (424 species in 251 genera), while basidiomycetes (12 species in 9 genera) and asexual fungi (94 species in 61 genera) are less abundant. Based on the number of fungi on average associated with each land plant in the United Kingdom, it is estimated that about 1.5 million fungal species exist, but only 70,000 species (less than 5%) have been described (Hawksworth 1991). Of those 70,000 species, 530 are higher marine fungi (0.76%). This suggests that, based on this ratio, there are a total of 9,450 species of higher marine fungi in existence; of which more than 8,900 have yet to be described. Additionally, this estimation does not take into account the higher fungal diversity that may exist in understudied subtropical and tropical

regions (Aime and Brearley 2012); Cannon (1997) suggest the existence of 10 million fungal species.

Adaptations

Marine ascomycetes have adapted to aquatic habitats in a variety of ways. The presence of ascospore appendages or mucilaginous sheaths allows attachment to substrates in moving water during passive ascospore dispersal (Kohlmeyer and Kohlmeyer 1979, Hyde and Jones 1989, Shearer 1993, Jones 1995). Appendages may be gelatinous (e.g., *Spathulospora*), rigid (e.g., *Halosphaeriopsis*, *Arenariomyces*), ribbon-like (e.g., *Corollospora*), thread-like (e.g., *Crinigera*), bristle-like (e.g., *Etheirophora*, *Dryosphaera*), strand-like (e.g., *Nimbospora*), or hair-like (*Capillataspora*). They may unfurl into long viscous thread-like structures (e.g., *Aniptodera, Halosarpheia*, *Ophiodeira*), have mucilage-filled end chambers *(*e.g., *Lulworthia, Linocarpon*, *Kohlmeyeriella*), or have sticky gelatinous sheaths (e.g., *Leptosphaeria*, *Massarina*, *Phaeosphaeria*, *Pleospora*, *Trematosphaeria*). Marine fungal spore shapes are also an adaptation to the marine environment. The sigmoid (e.g., *Kohlmeyeriella, Vibrissea*) and filiform (e.g., *Lindra*, *Lulworthia*) spore forms increase the area of orthogonal projection and the long filamentous structure increases the probability of entanglement with potential substrates. Adaptations to life in the intertidal zone include the ability to withstand the friction of sand particles, and intermittent dessication during low tide with concomitant exposure to high air temperatures and high UV irradiation. Thus the tough carbonaceous ascomata of *Corollospora* species may explain the ubiquity of this genus in intertidal environments worldwide.

Systematics

Recent molecular phylogenetic analyses have rejected the theory of a single evolutionary transition between marine and terrestrial ascomycetes (Spatafora et al. 1998). The majority of the obligate marine ascomycetes form a monophyletic clade, which represents the Halosphaeriaceae, a family with 58 genera in the monotypic order Halosphaeriales. Analyses demonstrated that the Halosphaeriaceae have evolved from a terrestrial ancestor. A second monophyletic clade is formed by the Lulworthiaceae, a family of six genera in the monotypic order Lulworthiales (Spatafora et al. 1998, Campbell et al. 2005). The Lulworthiales represent an independent transition from terrestrial to marine environments (Spatafora et al. 1998). Most recently, phylogenetic analyses have indicated a third clade of obligate marine ascomycetes, the Koralionastetaceae, a family of two genera in the monotypic order Koralionastetales, a sister clade to the Lulworthiales (Campbell et al. 2009). The genus *Pontogeneia* was described by Kohlmeyer (1975) and the seven species of *Pontogeneia* currently known parasitize marine Chlorophyta or Phaeophyta. The family Koralionastetaceae was proposed by Kohlmeyer & Volkmann-Kohlmeyer (1987) based on the single genus, *Koralionastes*, with three species from corals. Two species were added later by Kohlmeyer and Volkmann-Kohlmeyer (1990a). Koralionastetales differs from the other orders of the Sordariomycetes (Zhang et al. 2006) by the characteristic formation of antheridia on germinating ascospores.

Geographical Distribution

Marine and estuarine environments occupy 75% of the globe and include oceans, river mouths, tidal creeks and bayous. Most marine fungi require oxygen and all require

an organic substrate and thus they are found primarily in intertidal zones of the coastal ocean worldwide. Further, some species have been collected from the deep sea, with the deepest records at 5315 m for *Periconia abyssa* (Kohlmeyer 1977) and a set of culturable filamentous fungi obtained from deep-sea hydrothermal vents at depths ranging from 900 to 3630 m (Burgaud et al. 2009). The two most important factors in the geographical distribution of marine fungi are thought to be water temperature and salinity (Booth and Kenkel 1986), which is also the case for marine bacteria. Hughes (1974) divided the oceans into zones based on their average temperature range over the year. Consequently, species of marine fungi are classified as temperate, tropical or cosmopolitan (Hughes 1974, Kohlmeyer and Kohlmeyer 1979). Certain areas have been examined for marine fungi more thoroughly than others, thought to be due in part to the location and travel routes of marine mycologists; these areas include: India, China, Thailand, Egypt, Denmark, Australia, British Isles, Malaysia, Belize, and San Juan Island (Kohlmeyer 1983, Shearer et al. 2007). However, vast regions, including a large proportion of the U.S. coastline, remain unsampled.

Importance and Role

Marine fungi have often been ignored as participants in coastal ecological processes, although they play a vital role in the decomposition of organic matter, and carbon and nitrogen cycling. Marine fungi can drive mineral and energy cycling within marine ecosystems (e.g. nitrogen and phosphorus) and they can influence community composition within ecosystems through their interactions with other organisms – producing toxic or appealing secondary metabolites, as a food resource for invertebrates including zooplankton, and as pathogens or symbionts. As well, many studies are now

reporting bioactive metabolites from marine fungi, including novel antimicrobial, antiparasitic and anticancer compounds (Cuomo et al. 1995, Christophersen et al. 1998, Cueto et al. 2001, Chen et al. 2003, Rowley et al. 2003, Klemke et al. 2004, Tsuda et al. 2004, Lin et al. 2002, Bhadury et al. 2006, Ebel 2006)(Table 1).

Primarily saprotrophic, fungi in marine environments can also function as symbionts or parasites of marine plants and animals. Roles can change based on environmental conditions: a single fungal species may transition from marine plant endophyte to saprotroph when the plant dies (Porras-Alfaro and Bayman 2011).

A. Saprotrophs. Fungi are able to degrade a wide range of recalcitrant biological molecules and in recent years have been shown to play a significant degradative role in marine and estuarine environments (Newell 1996, Newell and Porter 2000, Kohlmeyer et al. 2004). Saprotrophic fungi break down cellulose, lignocellulose, agar, laminarin, chitin, keratin, tunicin and calcium carbonate and they occur on driftwood, intertidal wood, marsh plants, mangrove vegetation, algae and animal substrates (Kohlmeyer et al. 2004). They play a critical role in the decomposition process and provide a primary link in the remineralization and transformation of decaying material (Buchan et al. 2003).

The majority of marine fungal species have been described from wood, due to its availability as a substrate, and owing to the destruction of marine timber as an economic concern. Fungi play an important facilitative role by softening the surface of the wood, which then enables the larvae of wood-boring mollusks to penetrate the surface. Evidence has shown that these larvae preferably accumulate on wood that has been predigested" by fungi (Kohlmeyer and Kohlmeyer 1979). Boring crustaceans may also depend on marine

Table 1

Examples of secondary metabolites produced in fermentation broth from endophytic marine fungi (modified from Raghukumar 2008)

Host	Fungus	Secondary metabolite	Activity/application	Reference
Marine algae				
Red alga (*Actinotrichia fragilis*)	*Penicillium citrinum*	Alkaloid	Anti-cancer	Tsuda et al. 2004
Red alga (*Polysiphonia violacea*)	*Apiospora montagnei*	Diterpene	Activity against human cancer cell lines	Klemke et al. 2004
Green alga (*Codium fragile*)	*Fusarium* sp.	Cyclic tetrapeptide	Anti-cancer	Ebel 2006
Seagrasses				
Shoal grass *Halodule wrightii*	*Scytalidium* sp.	Hexapeptide	Inhibitor of *Herpes* simplex virus	Rowley et al. 2003
Mangroves				
Kandelia candel	Unidentified endophytic fungus	Lactones	Active against bollworm and parasitic copepods	Chen et al. 2003

fungi to digest wood. Studies have shown that *Limnoria* isopods can live on fungus-free wood but their lifespan is prolonged if fungi are present in the wood (Kohlmeyer and Kohlmeyer 1979). Additionally, *Limnoria* cannot reproduce if marine fungi are not present on the wood. The fungi may supply proteins, vitamins and oils which are thought to be necessary for reproduction (Kohlmeyer and Kohlmeyer 1979).

Marine fungi are the principle decomposers of salt marsh plants (Kohlmeyer and Volkmann-Kohlmeyer 2001). A large proportion of salt marsh ecosystem photosynthate is contained in the plant structural molecule lignocellulose. Other trophic levels depend on fungal extracellular enzymes to cleave lignocellulose into smaller molecular weight compounds that can enter the food web via mycophagic grazing invertebrates and bacteria (Newell and Porter 2000). Seventy to 75 percent of the organic mass of mature shoots of *Spartina alterniflora* is comprised of lignocellulose (Newell and Porter 2000), with similar values for *Juncus* species (78%) (Kuehn et al. 2000). As the major contributor to denitrification rates and mineralization of carbon and nitrogen (Lillebo et al. 1999), fungi are vital to salt marsh nutrient cycling processes. Salt marsh plants do not abscise their leaves, and decay occurs while the plants are still standing (Newell and Palm 1998). Marine fungi dominate the initial stages of decay (Newell 1996, Kohlmeyer and Volkmann-Kohlmeyer 2001); bacteria play a larger role in decomposition once the plants collapse onto the sediment during late decay (Newell and Palm 1998). Walker and Campbell (2010) found marine fungal communities establish in created salt marshes as early as one year after marsh creation. Mangrove vegetation is the tropical counterpart of temperate salt marshes and in the subtropics both vegetation types may overlap, although their fungal communities are thought to be distinct (Shearer et al. 2007).

Higher filamentous marine fungi are involved in the decomposition of algae along with bacteria and yeasts. Members of the Phaeophyta, such as *Sargassum* species, are the main algal substrate for saprotrophic marine fungi because the tough brown algae are more resistant to decay than Chlorophyta and Rhodophyta and permit the slower growing marine fungi to establish. The more chemically-labile green and red algae are decomposed by bacteria and yeasts before the marine fungi have established (Zuccaro and Mitchell 2005).

Marine fungi on animal substrates are restricted to exoskeletons, shells and protective tubes. Such substrates consist of chitin, keratin, tunicin and calcium carbonate with an organic matrix. Currently, the importance of fungi in the breakdown of such biopolymers in the marine environment is unknown and requires further study.

Similarly, the mycota of sand is not well characterized and the number of reported indigenous arenicolous fungi is small (Koehn 1982). Arenicolous fungi live among or on sand grains, degrading organic material between sand grains to obtain nutrition. At maturity, arenicolous fungi release their spores into the water where they are then trapped by air bubbles in sea foam (Kirk 1983). Seafoam contains only un-germinated spores and conidia. Constant movement prevents germination, thus spores do not germinate until they are washed up onto intertidal substrates such as algae and wood. Examination of seafoam for fungal spores is thought to provide a snapshot of fungal species present in the intertidal zone (Kohlmeyer and Kohlmeyer 1979). In littoral food webs, fungi are crucial decomposers of the recalcitrant components of organic matter that are indigestible to interstitial animals: cellulose and lignin from higher plants, alginates and laminarin from brown algae and agar from red algae. Interstitial animals such as mites (Oribatei)

have been shown to feed preferentially on fungal hyphae and spores (Kohlmeyer and Kohlmeyer 1979). Fungal mycelium also increases the permeability of the surface layer of sandy soils via bioturbation processes (Propp 2003).

B. Symbionts. Marine fungi form several types of symbiotic associations as marine lichens, in partnership with microscopic cyanobacteria or green algae. The fungus produces the characteristic lichen thallus structure, although the morphological form of the composite thallus is a result of the interaction between both partners (Will-Wolf et al. 2004). In facultative marine lichens each partner can occur in a free-living state. In obligate marine lichens the fungal partner cannot exist without the photobiont. Finally, mycophycobioses are obligate symbioses between systemic fungi and marine macroalgae in which the alga is the exhabitant and dominates the symbiosis (Kohlmeyer and Kohlmeyer 1979).

C. Pathogens. Fungal pathogens are important emerging threats to global biodiversity (Fisher et al. 2012). Forty species of higher marine fungi are known parasites and all but two of them parasitize algae (Kohlmeyer and Volkmann-Kohlmeyer 2003); one species parasitizes crab carapaces and the other mangrove prop-roots. The fungi that parasitize algae are specific to each algal class (Chlorophyta, Phaeophyta or Rhodophyta). Some are not restricted to genera within that class, but others are species specific. Fungal disease symptoms in algae cannot always be easily recognized and have been defined as a continuing disturbance to the plant's normal function or state such that the plant is altered in growth rate, appearance, or economic importance (Kohlmeyer and Kohlmeyer 1979).

The number of host plants or animals infected by fungi in marine environments is usually small (Kohlmeyer and Volkmann-Kohlmeyer 2003). An exception to this is the unusual case of a terrestrial fungus switching its host to a marine invertebrate, causing aspergillosis of Caribbean sea fan corals (Nagelkerken et al. 1997). The pathogen, identified molecularly as *Aspergillus sydowii* (Geiser et al. 1998), was previously known only as a soil-borne fungus causing opportunistic infections of terrestrial species. In sea fans (*Gorgonia* spp.), monitoring studies have shown that aspergillosis can rapidly erode the coral tissue and, in some cases, cause death. Although the genetic differences between the marine and terrestrial *A. sydowii* isolates were small, those isolates pathogenic to *Gorgonia* sea fans were physiologically and biochemically distinct (Alker et al. 2001). The emergence of *A. sydowii* as a marine pathogen points to the ineffectiveness of the land-sea boundary as a barrier to disease transmission (Harvell et al. 1999) and the ongoing loss of coral reefs is a current threat to marine ecosystem stability and function.

Pathogenic marine fungi may directly interact with humans as potential causal agents of disease in sand beaches. Sediments of five Ligurian beaches in compliance with European Union bathing water regulations were studied based on the characteristics of their fungal communities during the tourist season (Salvo and Fabiano 2007). Among the 179 taxa of filamentous fungi isolated, 120 were opportunistic pathogens, such as *Acremonium* sp., and *Penicillium citrinum*. Furthermore, 5% of the total filamentous fungi belonged to the dermatophyte genus *Microsporum*, a known causal agent of mycoses. Beach sediments showed elevated densities of opportunistic pathogens, of pathogenic filamentous fungi, and of pathogenic yeasts during the tourism season. Pathogenic beach fungi have received little attention and disease transmission from

sediments has not yet been demonstrated, however studies such as Salvo and Fabiano (2007) indicate beach sediments may act as a reservoir of potential pathogens, including fungi such as *Aspergillus niger*, causal agent of otomycosis (‘‘swimmer’s ear’’). Salvo and Fabiano (2007) found the number of pathogens detected increased with human beach use and it was concluded that fungal communities may prove a useful tool for assessing the quality of sand beach sanitation and environmental quality. In addition, Walker and Campbell (2008) inventoried non-pathogenic filamentous higher marine fungi at seven Mississippi Gulf Coast beaches ranging from lightly impacted (Horn Island) to heavily impacted by human activity (Gulfport) in 2008. Native saprotrophic fungal species richness decreased with increasing human presence, suggesting another potential role for fungi as indicators of beach health.

Although the majority of fungi in coastal habitats play beneficial roles, certain fungal species threaten coastal salt marshes and seagrass beds, impairing their function as nursery habitats and reducing their aesthetic appeal. *Fusarium* spp. have been implicated in salt marsh browning and die-back on the U.S. East and Gulf Coasts (Alber et al. 2008). Pathogenic isolates have been obtained, including a strain in Louisiana belonging to an African clade of *Fusarium*. This has led to speculation that African dust may have introduced the pathogen to the New World in a manner similar to that of coral reef disease (Garrison et al. 2003).

Fungi are considered to be opportunistic, weak pathogens of fish and crustaceans, problematic only when host defenses are already compromised, or during stressful conditions (Noga 1993), such as during captive rearing for aquaculture. *Fusarium solani*

causes black gill disease of crustaceans, a problem in marine shrimp ponds (Bian and Egusa 1981).

Applications

Fungal Single-Celled Protein (SCP) Production

Due to concerns over the sustainability of wild fisheries, as well as the increasing cost and restricted supply of fishmeal as a protein source for aquaculture-reared fish, it has become necessary to examine the substitution of fishmeal with an alternative protein source. The term "single-celled protein" (SCP) refers to protein obtained from dead, dry cells of microorganisms such as bacteria, fungi and algae. The use of SCP for animal feed has been in practice since 1920 (Litchfield 1989). In more recent years, advances in scientific knowledge of the physiology, nutrition and genetics of microorganisms have led to significant improvements in SCP production for both human consumption and animal feed (Israelidis 1988, Litchfield 1989). However, while the production of SCP for animal feed is abundant (Martin et al. 1993), it has not been utilized or studied widely for aquaculture feed. Fungal SCP is promising, as algal SCP has an unacceptable coloration and disagreeable flavor, high lipid content and lower nucleic acid profile than the FAO standard. As well, nitrogen availability is low as algae have a non-digestible cell wall. SCP from bacteria has a high nucleic acid content, but much of it is in the form of non-protein nitrogen which fish are unable to digest. In contrast, fungal SCP has a nucleic acid profile comparable to that of fish protein, amino acid concentrations higher than FAO standards, and low lipid content. Additionally fungi can utilize carbon from a vast range of substrates, including nontoxic industrial wastes such as spent brewery grain. Moreover, SCP from fungi is more acceptable to regulatory agencies and the public than

SCP from bacteria (Hulge 1995). Fungal species in polycultures (*Candida kruzei, Geotrichum candidum, Hansenula anomala* (now known as *Pichia anomala*); (Murray and Marchant 1986) and monocultures (*Candida utilis*; Martin et al. 1993) have performed well in fermentation trials and show promise for the scaling-up process required for use in commercial aquaculture (Campbell and Walker 2008).

Secondary metabolites

Due to their taxonomic diversity, heterotrophic lifestyle and extracellular digestion, fungi produce a wide variety of unique enzymes. These secondary metabolites can be harnessed for industrial, pharmaceutical and bioremedial purposes. An understanding of the adaptations of marine fungi found in association with marine algae, seagrass and mangroves, fungi cohabiting with marine invertebrates, especially corals and sponges, fungi in marine detritus, and fungi in extreme marine environments is likely to provide us with new insights into eukaryotic adaptation and potentially many new genes.

A. Industrial enzymes: Prawn waste, a chitinous solid waste of the shellfish processing industry, was used as a substrate for chitinase production by a marine-derived isolate of the fungus *Beauveria bassiana* using solid-state fermentation (Suresh and Chandrasekaran 1998). Fungi occurring in decomposing plant organic material or detritus in the sea have been shown to be a source of several wood-degrading enzymes of importance in paper and pulp industries (Raghukumar 2008). The deep sea, an extreme environment of high hydrostatic pressure and low temperature, hydrothermal vents with high hydrostatic pressure, high temperature and metal concentrations, and anoxic marine sediments are some of the unexplored sources of biotechnologically useful fungi.

B. Pharmaceuticals: Marine fungi represent a new area of interest for the bioprospecting of a wide variety of novel pharmaceutical compounds that can benefit human health. A new polyketide, the ortho-quinone obionin A, which aids in dopamine uptake in the brain, was isolated from the salt marsh fungus *Passeriniella obiones* (formerly *Leptosphaeria obiones*) (Fenical and Jensen 1993). A new antibiotic, auranticin A, was isolated from the mangrove fungus *Prussia aurantaica*, active against *Bacillus subtilis* and *Staphylococcus aureus* (Poch and Gloer 1991). A new antifungal diketopiperazine was isolated from an unknown fungal endophyte of laver (*Porphyra yezoensis*) (Byun et al. 2003). As well, new anticancer and antiviral molecules have been isolated from marine fungi (Table 1). It is likely that microorganisms including fungi may be the actual producers of many bioactive compounds reported from marine plants and animals (Raghukumar 2008). Currently, an estimated 8950 new marine fungal species await discovery and description, and with them, the strong possibility of many new bioactive molecules.

C. Marine bioremediation: Environmental contamination is an ongoing threat to coastal ecosystems and human health. Certain fungi can cleave the C-H bonds (Van Beilen et al. 2003) and C-C bonds (Harvey and Thurston 2001) in hydrocarbons, or sequester heavy metals (Stamets 2005). Fungi facilitate further degradation of contaminants by other organisms by making otherwise-inaccessible nutrients available from recalcitrant molecules. Many fungi can degrade high molecular weight aromatic compounds that are unavailable to bacteria (Cerniglia and Sutherland 2006). The numerous studies and patents (e.g. Stamets 2005, D'Annibale et al. 2006) generated by fungal bioremediation of terrestrial contaminants have led to the term *mycoremediation*

for this exciting new field, which is in its infancy in the marine environment. Over 72 fungal genera have demonstrated hydrocarbon degradation capability, with over 25 of these genera isolated from marine environments (Bartha and Atlas 1987, Bartha and Bossert 1984, Das and Chandran 2011). Many arenicolous fungal species of *Corollospora*, *Dendryphiella*, *Lulworthia* and *Varicosporina* can grow using alkanes and alkenes as their sole carbon sources and can mineralize n[1-^{14}C]hexadecane (Kirk and Gordon 1988).

A known white-rotting terrestrial basidiomycete fungus, *Flavodon flavus*, isolated from decomposing leaves of seagrass, decolorized pigments in molasses spent wash (MSW), a byproduct of ethanol distillation, by 80% after 8 days of incubation (Raghukumar and Rivonkar 2001). Decolorizing activity was also present in media prepared with seawater having a salinity of 15. In addition to color, total phenolics and chemical oxygen demand were reduced by 50% in MSW treated with *F. flavus*, suggesting its potential in the bioremediation of other effluents (Raghukumar and Rivonkar 2001). Extrapolating from the findings of D'Annibale et al. (2006), additional objectives of marine mycoremediation should include screening indigenous marine fungal strains for aromatic hydrocarbon-degrading and heavy-metal sequestering potential. Assessing feasibility of ex-situ bioaugmentation, the introduction of cultured microorganisms into the environment for the purpose of enhancing bioremediation of organic contaminants, should also be a priority.

Summary

Fungi from coastal and marine ecosystems are a neglected but significant component of marine biodiversity and nutrient cycling. While the economic impacts of

marine fungal pathogens are myriad and occasionally devastating, they are often overlooked or understudied. Concomitantly, the potential exists for great economic and human health benefits arising from future fungal biotechnological ventures, in realms such as single-celled protein production for aquaculture feed, pharmaceutical development and bioremediation. Perhaps most importantly, the existing detrital processing services of fungi in marine environments benefit coastal food webs via nutrient cycling, essential to ecosystem sustainability. The role of marine fungi in predictive ecosystem modeling and as biological indicators remains to be explored. When restoring coastal habitats, we must keep in mind the often invisible work of these key microbial players, which in addition to their ecosystem roles have the potential to heal us, feed us and detoxify marine pollutants.

Dissertation Research Justification

The current study will add to the existing body of knowledge for marine fungi in the following ways: knowledge of the occurrence and distribution of fungi will contribute towards a greater understanding of the role of these organisms in an understudied marine ecosystem: the intertidal zone of sand beaches. Regional inventories of economically important water bodies such as the U.S. Gulf of Mexico (GOM) are the first step in identifying the novel genetic potential of marine fungi. Knowledge of the factors influencing their distribution and community structure will aid in understanding their dispersal and colonization of marine habitats. The sampling frequency needed to adequately characterize the intertidal marine mycota for the U.S. GOM will be addressed, and the role of succession in obtaining species richness estimates during laboratory incubation will be determined.

This dissertation consists of four chapters and two appendices. This first chapter reviewed the current state of knowledge of marine fungi and outlines future research directions in basic and applied marine mycology. A flow chart (Figure 3) documents work undertaken during the course of this dissertation. Chapter II reports the results of the first inventory of marine ascomycetes of the U.S. GOM. New regional and host records are discussed for marine ascomycetes and differences in species richness by substrate are examined. Chapter III explores patterns of ascomycete diversity as they relate to latitude and season in Texas and Florida, based on ITS T-RFLP data of intertidal fungal communities on solid substrates (wood, emergent plant detritus, marine plant detritus). Finally, Chapter IV reports the findings of increased sampling frequency at two Mississippi beaches and outlines the optimized marine ascomycete collection strategy developed during this study. A summary of findings is provided to tie the four chapters together and provide direction for future work. The appendices contain additional information generated during the course of this study. Appendix A provides an annotated checklist of higher filamentous marine fungi reported from the GOM compiled from published research and this study. Appendix B provides physical-chemical data recorded at each collection site.

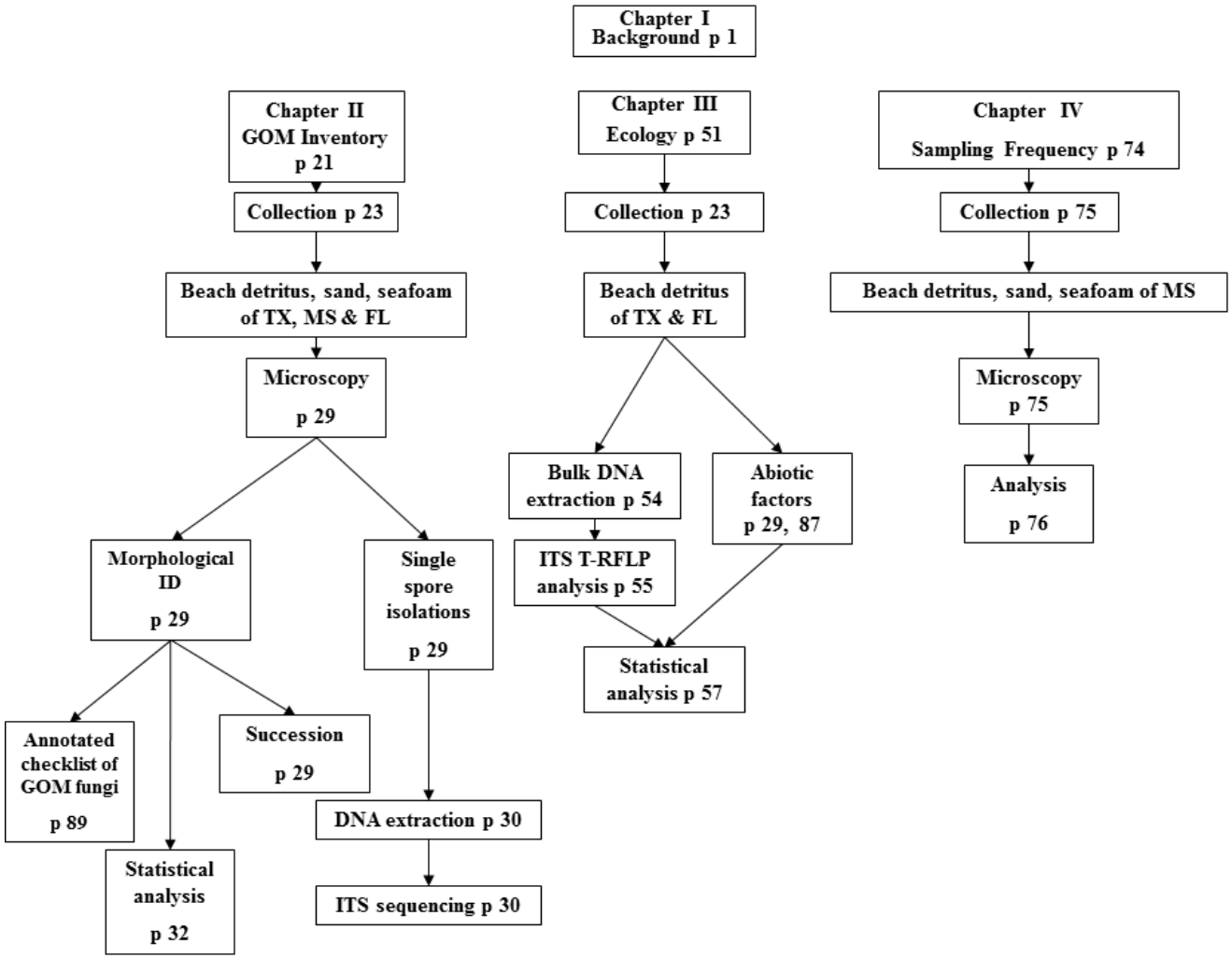

Figure 3. Flow chart documenting work undertaken during the course of this dissertation.

CHAPTER II

ASSESSMENT OF INTERTIDAL ASCOMYCETE DIVERSITY OF THE U.S. GULF OF MEXICO

Introduction

The U.S. Gulf of Mexico (GOM) is a mycologically understudied area and its marine mycota is largely unknown, although it encompasses warm-temperate and subtropical biogeographic zones. Previous mycological studies have shown that subtropical zones may contain a high diversity of cosmopolitan, temperate, sub-tropical and tropical marine fungal species (Kohlmeyer and Kohlmeyer 1979). Species of filamentous higher marine fungi have been summarized (Jones et al. 2009, González 2009), with most fungi isolated from marine environments thus far belonging to the Phylum Ascomycota. Prior to the current study, 251 ascomycete genera containing 424 species were known from marine environments globally (Jones et al. 2009), with 20 ascomycete genera and 42 species reported from the GOM (González 2009).

Ascomycete fungi are the principle decomposers of the dominant GOM emergent saltmarsh plants *Spartina alterniflora* (Fam. Poaceae), and *Juncus roemerianus* (Fam. Juncaceae) (Kohlmeyer and Volkmann-Kohlmeyer 2001, Walker and Campbell 2010). Seventy to seventy-five percent of the organic mass of mature shoots of *Spartina alterniflora* is comprised of the plant structural molecule lignocellulose and other trophic levels depend on fungal extracellular enzymes to cleave lignocellulose into smaller molecular weight compounds that can enter the food web via mycophagic grazing invertebrates and bacteria (Newell and Porter 2000). As the major contributor to denitrification and mineralization of carbon and nitrogen (Lillebo et al. 1999),

ascomycetes have an established and vital role in saltmarsh nutrient cycling. However, little is known about their role in decaying intertidal saltmarsh detritus and other marine detritus on sand beaches.

The marine fungal diversity of certain geographic areas has been explored more intensively than others, coinciding with the location of marine laboratories and the travel routes of marine mycologists (Kohlmeyer and Kohlmeyer 1979, Shearer et al. 2007, González 2009): British Isles, Hawaiian Islands, Caribbean Islands, San Juan Island, China, Australia, the Seychelles, India, Denmark and Japan. However, in spite of the importance of marine fungi, no thorough inventory of marine biodiversity has been carried out in one any area (Kohlmeyer et al. 2004). Recently, a number of undescribed aquatic fungal taxa have been discovered during biodiversity studies in freshwater environments (Jeewon et al. 2003, Pinnoi et al. 2006, Raja and Shearer 2006, Vijaykrishna et al. 2006), and additional undersampled habitats such as the U.S. GOM represent a potential source of new taxa and genetic diversity. The GOM is the ninth largest body of water in the world and North America's most economically productive ecosystem (Tunnell 2009), spanning 2703 km of coastline, and receives freshwater and detritus from 33 major rivers that drain 31 states (Dawes et al. 2004).

To improve our knowledge of the biodiversity and geographical distribution of marine fungi, a biodiversity inventory of the U.S. GOM was conducted at selected intertidal barrier island beach sites in Texas, Mississippi and Florida. The objectives of this study were: 1) to characterize the marine fungal communities found on different intertidal substrates on GOM barrier island sand beaches; 2) to compare differences and similarities among the fungal communities associated with each substrate type; and 3) to

determine if ascomycete species succession occurs during 3-12 month substrate incubation, and if so, to examine the effect of succession on species richness estimates.

Materials and Methods

Description of Study Sites

Collecting sites (n=8) were located along the U.S. GOM coastline (Figure 4, Table 2). The mean annual precipitation for the continental Gulf Coast is 167.6 cm; the mean annual temperature is 20°C, with a summer average temperature of 35°C, and a winter average minimum of 6.7°C. Beach intertidal sites were chosen to encompass latitudinal and longitudinal gradients to ensure the greatest diversity of marine fungal species would be encountered (Kohlmeyer et al. 2004). Most sites were located on barrier islands to minimize the effects of human disturbance on species diversity, as it has been shown that human impacts decrease the frequency of occurrence and diversity of marine fungal species present (Walker and Campbell 2008). In addition to sites in Texas and Florida, the inclusion of West Ship Island, MS as a collection site allowed this study biogeographic coverage of all regions of the U.S. GOM (southwest, northwest, northcentral, northeast and southeast).

Texas has a 1004-km long Gulf of Mexico coastline. Salt marshes dominate the emergent coastal vegetation in the north, while Black Mangrove (*Avicennia germinans*) fulfills a similar ecological role in the south (Moulton et al. 1997).

Site 1. Padre Island TX. Padre Island is 185 km long and contains the longest sand beach in the United States. Padre Island provides important habitat for marine and terrestrial plants and animals, including a number of rare, threatened, and endangered species. The Laguna Madre, one of the few hypersaline lagoon environments in the

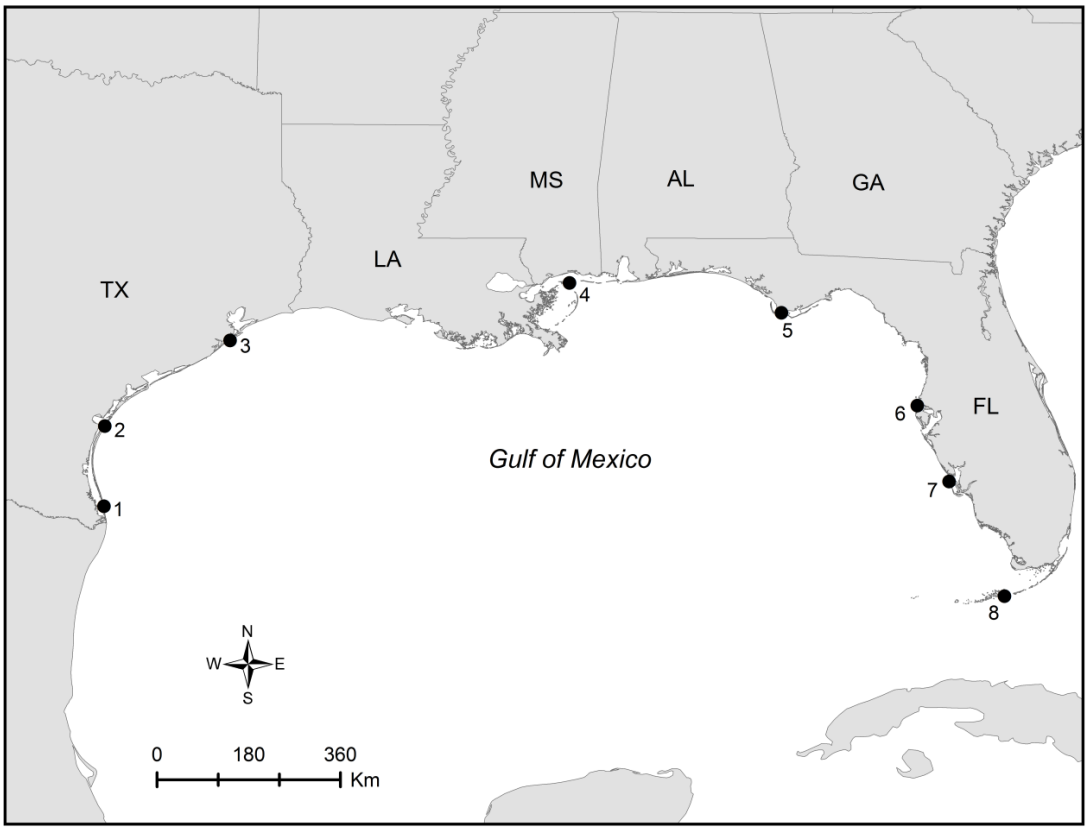

Figure 4. Intertidal collection sites of the United States Gulf of Mexico (Texas, Mississippi and Florida).

Table 2

GPS coordinates of intertidal collection sites in Texas, Mississippi and Florida

	N	W
TEXAS		
1. Galveston Island State Park, Galveston Co.	29°11'20.90"	94°57'32.10"
2. Mustang Island State Park, Nueces Co.	27°40'14.80"	97°10'12.30"
3. Padre Island, Cameron Co.	26°14'52.00"	97°11'05.10"
MISSISSIPPI		
4. West Ship Island, Harrison Co.	30°12'27.52"	88°57'49.24"
FLORIDA		
5. St. Vincent National Wildlife Refuge, Franklin Co.	29°40'36.00"	85°13'15.30"
6. Caladesi Island State Park, Pinellas Co.	28°02'03.80"	82°49'19.40"
7. Cayo Costa State Park, Lee Co.	26°41'17.90"	82°15'29.50"
8. Bahia Honda State Park, Monroe Co.	24°39'20.50"	81°16'47.80"

world, is situated between the western side of the island and the mainland, and is noted for the large numbers of waterfowl using the lagoon as a sanctuary and breeding ground.

Site 2. Mustang Island TX. Mustang Island is a 40-km long barrier island located near Corpus Christi. It totals 3,954 acres with 29 km of beach. Mustang Island has a unique and complicated ecosystem, dependent upon sand dunes anchored by sparse mats of drought-resistant vegetation: Sea Oats (*Uniola paniculata*), Beach Panic Grass (*Panicum amarum*) and Soilbind Morning Glory (*Ipomoea pes-caprae*). Large numbers of waterfowl and shorebirds are common. Mustang Island State Park is located on the southern end of the island.

Site 3. Galveston Island TX. Galveston Island is positioned on the natural harbor of Galveston Bay and has 51 km of sand beaches. It is a heavily developed island but maintains some of its natural beach habitat at Galveston Island State Park, located on the west side of the island, where collections were taken for this study.

Site 4. West Ship Island, MS. West Ship Island, part of the Gulf Islands National Seashore, is an undeveloped barrier island with 9.5 km of sand beach located 18 km south of Gulfport, Mississippi and is accessible only by boat. In 1969 Hurricane Camille cut Ship Island in two, creating East and West Ship Islands, which were further separated by Hurricane Katrina in 2005. Although the western portion of West Ship Island is a tourist destination from late March to early October, much natural vegetation and wildlife habitat remains.

Florida barrier island sites were included for several reasons. Based on climate and biotic data, Florida is a biotic transition zone between the warm temperate and subtropical zones (Henry et al. 1948) and is a known area of high biodiversity for many

organisms (Whitney et al. 2004). Therefore, its geographical location provides a unique region to investigate hypotheses about the role of latitude in structuring marine fungal communities and biogeographic distribution. Four collection sites were established in Florida for this study.

Site 5. St Vincent Island FL. St Vincent Island is an undeveloped barrier island with no human habitation near Port St. Joe in the Florida Panhandle. It is one of the largest northern GOM barrier islands and is home to the St. Vincent National Wildlife Refuge.

Site 6. Caladesi Island FL. Caladesi Island is an uninhabited and undeveloped barrier island near Tampa, Florida accessible only by boat. The island was created in 1921 when the Tarpon Springs Hurricane separated it from what was then Hog Island. The habitat of the island consists of virgin slash pine (*Pinus elliottii*) forest, sabal palm (*Sabal palmetto*) and saw palmetto (*Serenoa repens*), mangrove swamps comprised of Red Mangrove (*Rhizophora mangle*), Black Mangrove (*Avicennia germinans*) and White Mangrove (*Laguncularia racemosa*), and 5 km of undeveloped beaches with sand dunes covered with Sea Oats, Beach Morning-Glory and Sea Purslane (*Sesuvium portulacastrum*).

Site 7. La Costa Island FL. La Costa Island is an undeveloped barrier island near Fort Myers, Florida and is accessible only by boat. The island houses Cayo Costa Island State Park and Pine Island National Wildlife Refuge. The habitat consists of 14.5 km of sand beaches and acres of slash pine forests (*P. elliottii)*, live oak (*Quercus virginiana*) - palm hammocks and mangrove swamps comprised of Red, Black and White Mangroves (*R. mangle, A. germinans*, and *L. racemosa*, respectively).

Site 8. Bahia Honda State Park FL: While not a barrier island, this section of the Florida Keys has several km of undeveloped beach. Unlike the other sand beach sites sampled during this study, the geological formation of Bahia Honda is Key Largo limestone derived from a prehistoric coral reef similar to the present day living reefs of the Keys. Due a drop in sea level several tens of thousand years ago, portions of this ancient reef emerged from the sea, forming islands. Bahia Honda is the southernmost key where this formation is exposed and is unique in the Keys as it has extensive "sand" beaches (actually crushed limestone), as well as subtropical marine plant and animal species. Sampling site characteristics are summarized in Table 3.

Table 3

Sampling site characteristics that may influence marine fungal substrate availability. SAV = submerged aquatic vegetation

Site	Developed (D)/ Undeveloped (UD)	No. km sand beach	Saltmarsh/mangrove habitat present at collecting site	SAV/algal beds present at collecting site
1	D	112	No, but 1,825 km^2 coastal wetlands in nearby Laguna Madre	No, but 773 km^2 SAV in nearby Laguna Madre
2	D	29	No	SAV
3	D	51	No	No
4	UD	9.5	No	No
5	UD	18	No	No
6	UD	5	mangrove	No
7	UD	14.5	mangrove	No
8	UD	4	No	SAV

Sampling Strategy

Substrates were collected from the intertidal zone at each site once in winter (Dec 2008-Feb 2009) and once in summer (July-Sept 2009), with six months between each collection. West Ship Island, MS was sampled every other month for 10 months (April

2009-January 2010) to investigate changes in the intertidal fungal assemblage structure over shorter time scales (see Chapter IV).

Intertidal substrates were collected from the GOM side of each island along a 1-km transect on a falling tide. Replication was as follows for each transect based on substrate availability: seafoam (n=20), sand (n=10), marine plant detritus (algae/seagrass) (n=5), emergent plant detritus (salt marsh/mangrove) (n=5), and driftwood (n=10). Efforts were made to collect solid substrates that had been submerged in seawater for a considerable amount of time, as evidenced by presence of decay and colonization by other marine organisms such as barnacles. Solid substrates were placed in sterile plastic zippered bags with a paper towel dampened with sterile seawater and transported on ice back to the laboratory. Additional intertidal substrates (coconuts, shells, feathers, crustaceans, worm tubes, urchins, bryozoans, corals, sponges) were collected as available. Seafoam and sand were collected with a spoon-type skimmer and placed in sterile 50-mL conical tubes on ice. Upon returning from the field, subsamples of abundant substrates were immediately transferred to –20°C for future DNA extraction. Microscope mounts of seafoam, and seafoam stained with lactophenol cotton blue, were made immediately upon returning to the laboratory; remaining seafoam was stored at 4°C. Seafoam was examined directly under the microscope for the presence of fungal spores, as it has been reported to give a snapshot of the intertidal fungal assemblage (Kohlmeyer and Kohlmeyer 1979). Solid substrates were transferred to sterile plastic boxes containing paper towels, misted with artificial seawater (salinity 15) and incubated at room temperature (about 24°C) under natural day/night lighting conditions for 3-12 months depending on length of substrate decay period, with twice-weekly misting. All

TX and FL collections were examined for the presence of fungi within one week of collection, and periodically over 3-12 months (Shearer 1993, Shearer et al. 2004) to investigate whether fungal saprotroph succession occurred under laboratory incubation conditions. MS collections were examined within one week of collection (no successional data collected). Environmental data collected at each site (water temperature in °C, salinity, pH) are given in Appendix A.

Identifications

Marine ascomycetes were isolated from seafoam, sand and decaying beach detritus and identified morphologically using light microscopy to directly observe fungal reproductive structures. Ascomata were removed from solid substrates with a flame-sterilized needle, squash-mounted in sterile distilled water or lactophenol cotton blue, and examined using a NIKON Eclipse 80 microscope with Nomarski interference contrast optics. Dichotomous and pictorial keys (Kohlmeyer and Volkmann-Kohlmeyer 1991, Hyde and Sarma 2000) and relevant literature were employed for identification based on reproductive structures. Digital photographs of microscopic fungal structures were taken using a SPOT Insight camera and measurements (μm) were made using SPOT 4.1 software. Fungal succession was observed as changes in fungal assemblage composition (species presence/absence) based on morphological identifications from each substrate over time during 3-12 month laboratory incubation.

Molecular identifications were performed as follows: fungal isolates were cultured from decaying beach detritus onto antibiotic saltwater agar (ASWA) with a salinity of 35 (45 g Instant Ocean, 18 g agar, 250 mg streptomycin sulfate, 250 mg penicillin G, 1 L distilled H_20) and incubated at room temperature. Dilution plating of seafoam was carried

out using AWSA plates following standard procedures (Kohlmeyer et al. 2004) and incubated at room temperature. Plates were checked for growth once daily and new fungal colonies were subcultured to potato dextrose agar (PDA) to obtain single spore isolates. Fungal isolates on PDA and culture slants on saltwater potato dextrose agar (SWPDA) were maintained at 4°C in the dark.

DNA was extracted from single spore isolates using a Qiagen DNeasy Plant Mini Kit with the addition of lyticase (Raja et al. 2003). The non-coding internal transcribed spacer (ITS) region of fungal ribosomal DNA was targeted for polymerase chain reaction (PCR) amplification due to its highly conserved nature and high sequence variability at the species level (Larena et al. 1999). The majority of marine fungi are members of the phylum Ascomycota (Kohlmeyer et al. 2004) and thus PCR primers specific for ascomycetes were used. DNA was amplified using fungal ITS primers ITS 1-F: 5’ CTT GGT CAT TTA GAG GAA GTA A 3’ (Gardes and Bruns 1993) and ITS 4-A: 5’ CGC CGT TAC TGG GGC AAT CCC TG 3’ (Larena et al. 1999) (Invitrogen). This primer set amplifies a total product of 1225 base pairs (bp), which included the complete ITS region [ITS 1 and 2, and 5.8S (583 bp)], as well as the 3’ end of the 18S and the 5’ end of the 28S rDNA. PCR was performed using the following thermocycler parameters: a 3 min initial denaturation step at 95°C, followed by 35 cycles of 1 min at 95°C, 30 s annealing at 52°C and 1 min extension at 72°C, ending with a 10 min final extension at 72°C in a Thermo Electron Px2 thermal cycler. PCR products were purified using a QIAquick PCR purification kit (Qiagen). All samples were sequenced at the University of Illinois Urbana-Champaign Core Sequencing Facility using an Applied Biosystems 3730xl DNA Analyzer. Raw sequences were edited, contiguous DNA segments were assembled, and

consensus sequences were exported using Sequencher 4.7 (Gene Codes Corporation). Sequences obtained were then compared with the reference sequence database NCBI GenBank using the BLAST search engine (Altschul et al. 1997).

For the morphological presence/absence data, a site by species matrix was constructed. Within this matrix, substrates within sites were the column variables and taxa were the row variables. Each cell contained "1" if a taxon was present at that site and "0" if it was not. The matrix contained records of all the occurrences by site of marine fungal taxa found during this study. Fungal community differences due to latitude and longitude were observed as differences in fungal species presences and absences on the same substrate types at different locations. Percent species occurrence was calculated as follows: (No. collections of a sporulating species/Total no. samples collected supporting sporulating fungi) x 100. Species richness was adjusted to sampling effort by dividing the number of ascomycete species identified by the number of samples collected for each substrate type.

For each substrate type a rarefaction curve (number of species x number of samples) was plotted based on data from all 8 sites pooled to determine the number of samples required to characterize the mycota of each substrate type. Alpha and gamma diversity were calculated for each site/region based on morphological species identifications. Relative frequency of occurrence was calculated for each species. A two-way ANOSIM was performed in PRIMER v6.1.6 (Clarke and Gorley 2006) to assess the role of substrate and season in structuring fungal community differences. The role of latitude (site) is analysed and discussed in Chapter III.

Results

Seven hundred and fifty collections of beach detritus, sand and seafoam were made during the course of this study, with 288 of these collections (38.4%) supporting sporulating fungi. A total of 350 fungal isolates were made; 70% of which were identified and classified as 37 species of ascomycete fungi, while another 30% were morphologically unidentifiable. Of the species identified to genus, 30% were anamorphic (asexual) ascomycetes and 70% were sexual ascomycetes. Taxonomic composition of the total GOM fungal assemblage documented is given in Figure 5. Of the fungal taxa identified, 60% were found in only one collection (i.e. one sample from one site).

Twenty-seven species of ascomycete fungi were identified morphologically from decaying intertidal beach detritus, one from sand and 11 from seafoam, for a total of 37 discrete species. Frequency of occurrence of each marine ascomycete species that could be identified to genus, along with substrate information is given Table 4. In addition, a new location record of the protistan seagrass parasite *Plasmodiophora diplantherae* in detrital *Halodule wrightii* from Florida is noted in Table 4.

Examples of asci and ascospores (identification features) of marine ascomycetes collected from salt marsh detritus during this study are illustrated in Figure 6. An annotated checklist of marine fungi of the GOM, compiled from literature and this study, is given in Appendix B.

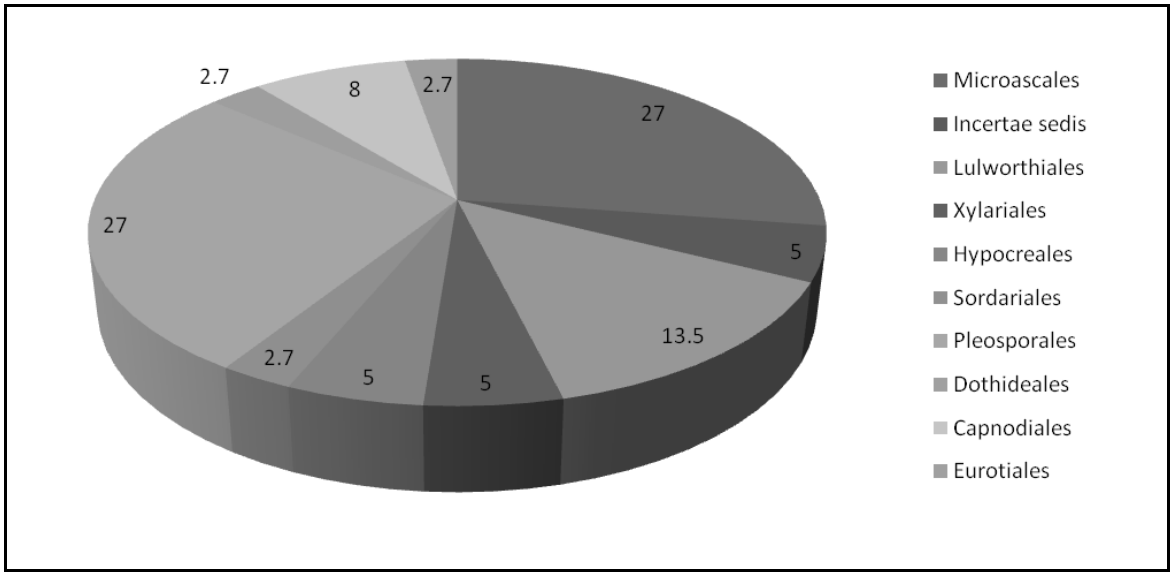

Figure 5. Percentage of total taxonomic composition of ascomycetes by Order, identified morphologically in 288 samples. Class Sordariomycetes: Hypocreales, Lulworthiales, Microascales, Xylariales; Class Dothideomycetes: Capnodiales, Dothidiales, Pleosporales; Class Eurotiomycetes: Eurotiales.

Sand

Corollospora maritima was the only fungus identified from sand during this study. This fungus was present at all collecting sites and identified from a variety of detrital substrates in addition to sand: seafoam, salt marsh, seagrass, *Sargassum*, *Ulva lactuca*, detrital wood, coconut, and worm tubes.

Seafoam

Spores of the following ascomycetes were identified microscopically via direct staining of seafoam with lactophenol cotton blue: *Corollospora maritima*, *Corollospora* sp., *Haloguignardia decidua*, *Pleospora* sp., *Alternaria* sp., *Lindra thalassiae,* and *Lindra* sp. 2. Of the twenty 50-mL seafoam samples collected per site, no more than 3 yielded fungal spores when examined microscopically. The most frequently isolated

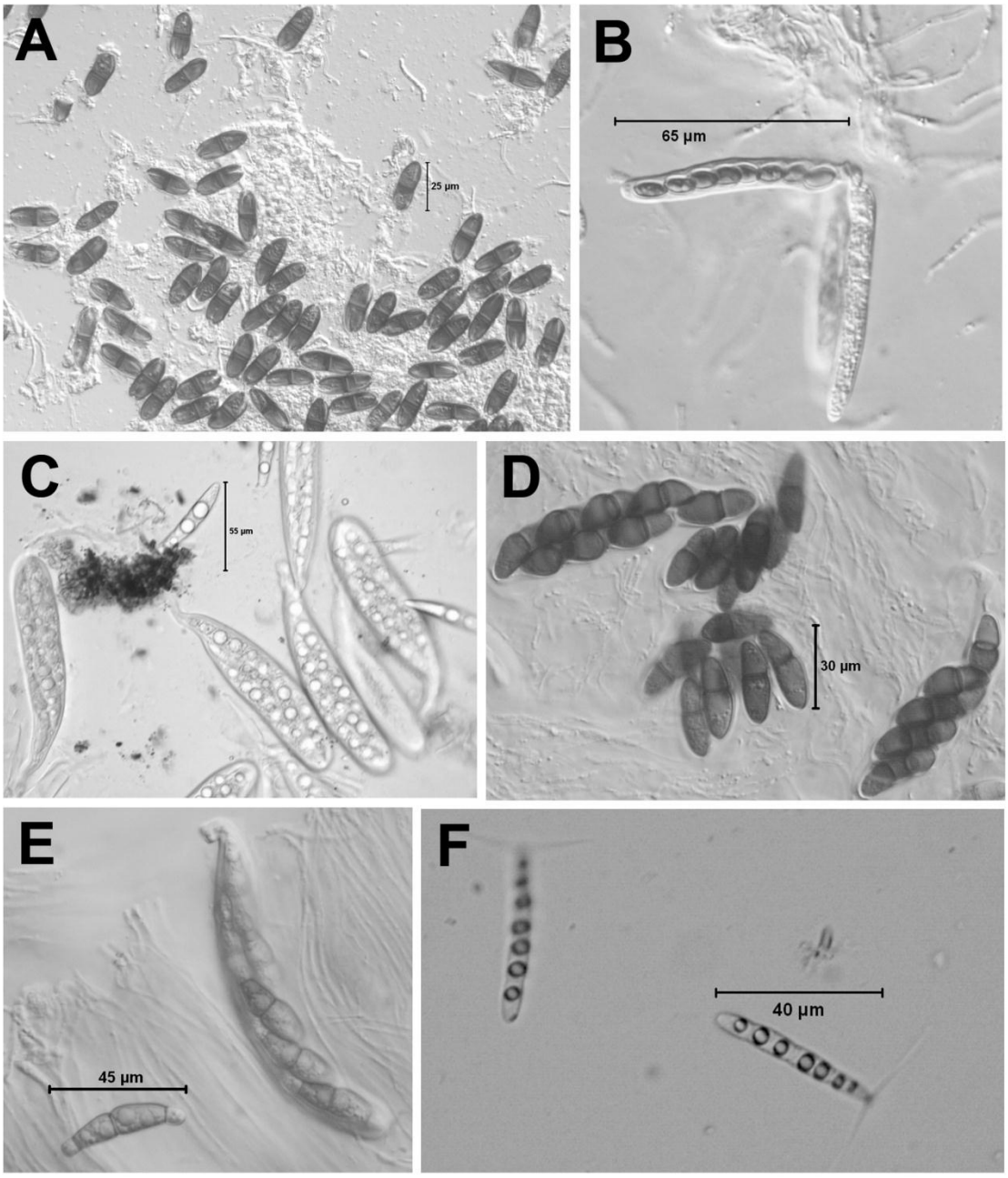

Figure 6. Examples of intertidal saprotrophic ascomycetes of the U.S. Gulf of Mexico collected from emergent plant (salt marsh) detritus. A. Ascospores of *Atrotorquata lineata* (400X). B. Asci and ascospores of *Anthostomella poecila* (400X). C. Asci and ascospores of *Loratospora aestuarii* (400X). D. Ascospores of an unidentified species of *Mycosphaerella* (400X). E. Ascus and ascospores of *Passeriniella obiones* (400X). F. Ascospores of *Torpedospora radiata* (400X).

fungi from seafoam, as recovered by direct plating of seafoam, and identified by ITS rDNA sequencing, were *Cladosporium* sp., *Alternaria tenuissima, Cochliobolus hawaiiensis*, and *Acremonium alternatum*. The sampling curve was not saturated for seafoam, and in 460 collections of seafoam only 12 fungal taxa were detected (Figure 7).

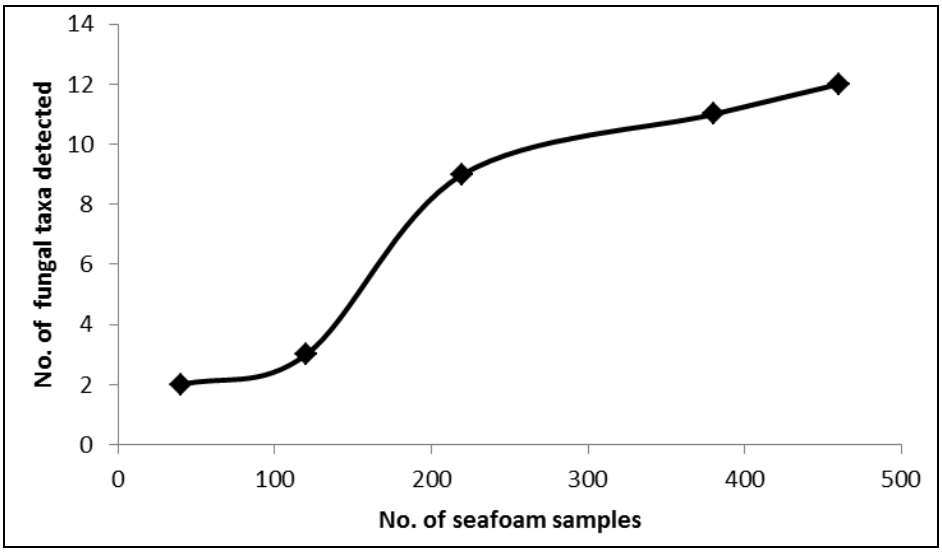

Figure 7. Relationship between the number of fungal taxa detected in seafoam and the number of seafoam samples collected.

Emergent plant detritus

Emergent plant detritus had the highest species richness (22 taxa) of any substrate sampled. The following five ascomycete taxa were found on *Spartina alterniflora* detritus: *Buergenerula spartinae*, *Mycosphaerella* undescribed species I, *Mycosphaerella* undescribed species II, *Phaeosphaeria halima*, *Phaeosphaeria spartinicola*. The following four ascomycete taxa were found on *Juncus roemerianus* detritus: *Anthostomella poecila, Massarina ricifera, Phaeosphaeria olivaceae* and *Phaeosphaeria roemeriani*. The following 12 taxa were found on unidentifiable saltmarsh plant detritus: *Atrotorquata lineata*, *Corollospora maritima*, *Loratospora aestuarii*, *Massariosphaeria typhicola*, *Passeriniella obiones*, *Pleospora pelvetiae*, *Torpedospora radiata*, *Periconia*

sp, *Tubercularia pulverulenta*, *Varicosporina ramulosa* and two unidentified coelomycetes. The sampling curve appears saturated around 70 samples for this substrate type (Figure 8)

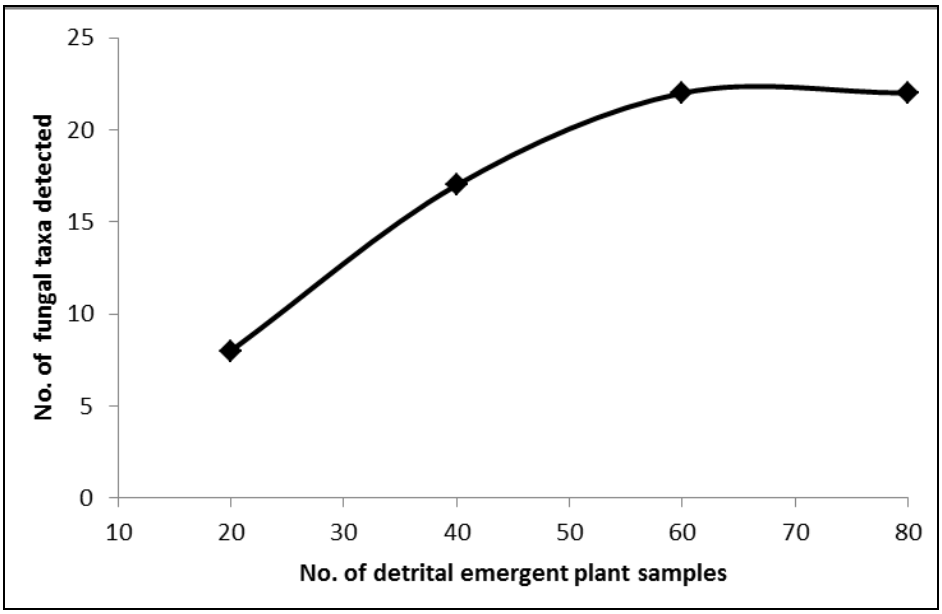

Figure 8. Relationship between the number of fungal taxa detected in emergent plant detritus and the number of emergent plant detrital samples collected.

Marine plant detritus

Eight fungal taxa were detected morphologically on marine plant detritus and all belonged to the genus *Corollospora* or its anamorphic genus *Varicosporina*. The following three species were found on detrital seagrass: *Corollospora maritima*, *Corollospora* species 3 and *Varicosporina ramulosa*. The following six species were found on detrital marine algae: *Corollospora gracilis*, *Corollospora intermedia*, *C. maritima*, *Corollospora* species 1, *C.* species 3, *Varicosporina prolifera* and *V. ramulosa*. The sampling curve appeared saturated around 30 samples for marine plant detritus (Figure 9).

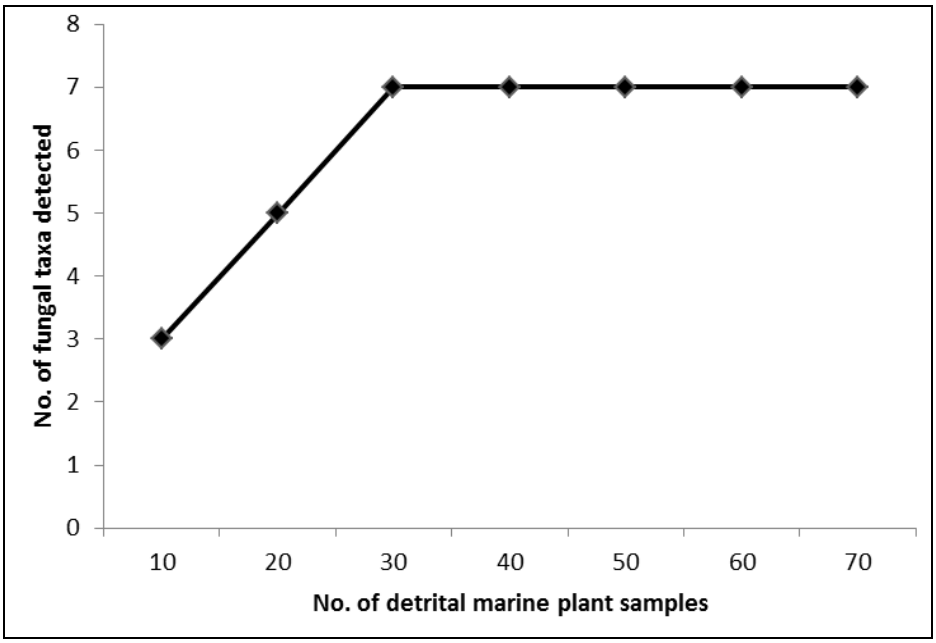

Figure 9. Relationship between number of fungal taxa detected and number of detrital marine plant samples collected.

Wood

The following 12 fungal taxa were identified from detrital wood: *Acrocordiopsis patilli*, *Alternaria* sp., *Corollospora maritima, Haiyanga salina, Leptosphaeria avicenniae, Lindra thalassiae, Lindra* sp. 2, *Passeriniella obiones*, *Sphaerulina oraemaris*, *Trichocladium achrasporum*, *Varicosporina ramulosa*, *Zalerion maritima*. The accumulation of species found on wood slowed at around 130 samples, indicating approximately this many samples may be required to characterize this substrate type morphologically (Figure 10).

A two-way ANOSIM and post-hoc testing for significant groups revealed a significant seasonal effect on the number of species per sample for marine detritus only ($R=0.353$, $P< 0.05$)(Figure 11).

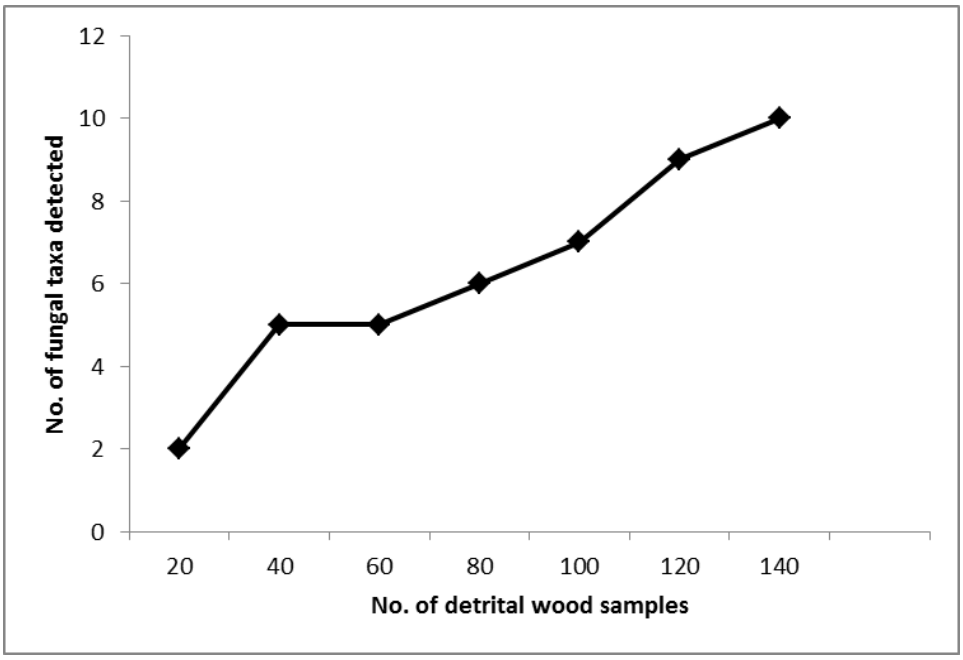

Figure 10. Relationship between the number of fungal taxa detected and the number of detrital wood samples collected.

Alpha diversity for each site based on morphological species identifications was as follows: in summer, site 1=3, site 2=2, site 3=2, site 4=12, site 5=2, site 6=3, site 7=4, site 8=2; in winter, site 1=3, site 2=7, site 3=2, site 4=8, site 5=4, site 6=4, site 7=2, site 8=5, with August and January collections used as summer and winter collections for Mississippi (West Ship Island). Regional species richness (gamma diversity) in summer based on morphological species identifications was as follows: Texas S=6, Mississippi S=12 and Florida S=7. Gamma diversity in winter for Texas was S=9, Mississippi S=8 and Florida S=8.

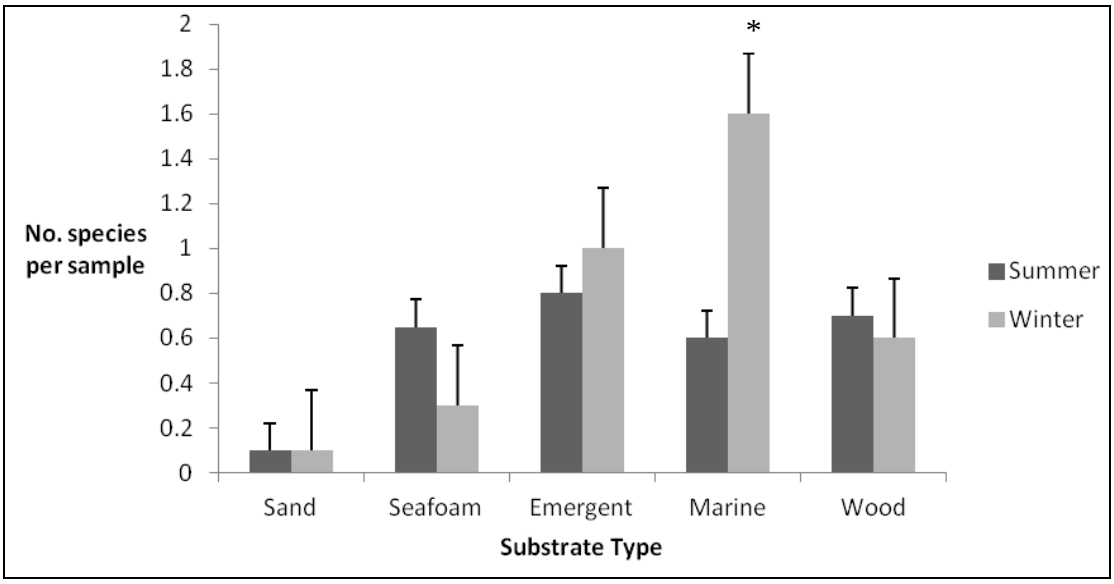

Figure 11. Marine fungal species richness as detected morphologically, by substrate and season (standardized to sampling effort). Significant differences in species richness by substrate type are denoted by an asterisk (*). Error bars represent standard error.

Discussion

Several of the fungal species detected during this study deviated from published descriptions, or were new records for the substrate or region. Three potentially new species of *Corollospora*, and two potentially new species of *Lindra* were collected. Nakagiri and Tokura (1987) described seven new *Corollospora* species from sand beaches of Japan. A new species from calcareous material associated with the seagrass *Zostera marina* and another from the shell of a shipworm in decayed driftwood, both from Egypt (Abdel-Wahab et al. 2009) bring the total to 20 currently described species of *Corollospora*. The present study documented three unidentified species of *Corollospora* from four different substrates (*Sargassum* sp., seafoam, *Halodule wrightii* and green alga, respectively) at three collecting sites in the GOM: South Padre Island (Site 1), West Ship Island (Site 4), and Caladesi Island (Site 6). These three species did not match any published *Corollospora* species description, based on ascospore and ascospore appendage

morphology. Additionally, two undescribed species of *Lindra* were collected, one from seafoam at South Padre Island (Site 1) and one from detrital wood at East Beach, MS, both with shorter ascospores than any other published *Lindra* species.

First described from *J. roemerianus* in North Carolina (Kohlmeyer et al. 1997), *Phaeosphaeria olivacea* is reported here for the first time from the GOM from West Ship Island MS (Site 4), on intertidal detrital *J. roemerianus*. From detrital wood, *Acrocordiopsis patilii* at Bahia Honda FL (Site 8) and *Haiyanga salina* at Caladesi Island FL (Site 6) were new records for the GOM, previously reported from mangrove wood from India and Brunei (*A. patilii*) and Hong Kong and Thailand (*H. salina*), respectively (Borse and Hyde 1989, Pang et al. 2008a). *Haiyanga salina* is a tropical to subtropical warm water species (Pang et al. 2008b).

Fungi identified morphologically in this study primarily belonged to classes Sordariomycetes or Dothideomycetes (Phylum Ascomycota), which represent a wide range of ecologies including pathogens and endophytes of plants, animal pathogens and mycoparasites. Dothideomycetes are pathogens, endophytes or epiphytes of living plants and also saprotrophs degrading cellulose and other complex carbohydrates in dead or decaying plant matter. Combinations of these niches can be occupied by a single fungus during its life cycle; for example several fungi begin their life cycles on living plants and switch to saprotrophic states when the plant dies or leaves are lost (Schoch et al. 2006). Nutritional modes of Dothideomycetes are not limited to associations with plants and several species are lichenized, while others occur as animal parasites or mycoparasites (Schoch et al. 2006).

Table 4

List of ascomycete taxa and one protistan marine slime mold detected morphologically on intertidal substrates of the U.S. Gulf of Mexico by site. Relative frequency of occurrence of fungal taxa in all collections housing sporulating fungi (n=288) as detected morphologically. For location of sites 1-8, see Table 2. EB = East Beach, MS

Species	Site 1	2345678EB	Detrital substrate	Relative frequency in all collections (%)
Sexual ascomycetes				
Acrocordiopsis patilii Borse & K.D. Hyde		+	Wood	0.35
Anthostomella poecila Kohlm., Volkm.-Kohlm. & O.E. Erikss.		+	*J. roemerianus*	3.5
Atrotorquata lineata Kohlm. & Volkm.-Kohlm.		+	Emergent	0.35
Buergenerula spartinae Kohlm. & R.V. Gessner		+	*S. alterniflora*	6.25
Corollospora gracilis Nakagiri & Tokura	+		*Sargassum*	0.35
Corollospora intermedia E.B.G. Jones	+		*Sargassum*	0.35
Corollospora maritima Werderm.	+	++++++++	Emergent, Marine, Seafoam, Wood, Sand, Shells, Worm tubes	22.6

Table 4 (continued).

Species	Site 1	2	3	4	5	6	7	8	EB	Detrital substrate	Relative frequency in all collections (%)
Corollospora pulchella Kohlm., I. Schmidt & N.B. Nair			+							Seafoam	0.35
Corollospora sp. 1	+									*Sargassum*	0.35
Corollospora sp. 2			+							Seafoam	0.35
Corollospora sp. 3				+						*H. wrightii*, green alga	0.69
Haiyanga salina (Meyers) K.L. Pang & E.B.G. Jones				+						Wood	0.35
Haloguignardia decidua Cribb & J.W. Cribb							+			Seafoam	0.35
Leptosphaeria avicenniae Kohlm. & E. Kohlm.				+						Wood	0.35
Lindra thalassiae Orpurt, Meyers, Boral & Simms							++			Seafoam, Wood	0.69
Lindra sp. 1 I.M. Wilson										Wood	0.35
Lindra sp. 2 I.M. Wilson	+									Seafoam	0.35
Loratospora aestuarii Kohlm. & Volkm.-Kohlm.							+			Emergent	0.35
Massarina ricifera Kohlm., Volkm.-Kohlm. & O.E. Erikss.							+			*J. roemerianus*	13.8
Massariosphaeria typhicola (P. Karst.) Leuchtm.							+			Emergent (salt marsh)	0.35

Table 4 (continued).

Species	Site 1	2	3	4	5	6	7	8	EB	Detrital substrate	Relative frequency in all collections (%)
Mycosphaerella sp. I Johanson									+	Emergent	2.8
Mycosphaerella sp. II Johanson									+	Emergent	0.35
Passeriniella obiones (P. Crouan & H. Crouan) Hyde&Mouz									+	E, Wood	11.11
Phaeosphaeria halima (T.W. Johnson) Shoemaker & C.E. Babc.									+	*S. alterniflora*	6.25
Phaeosphaeria olivacea Kohlm., Volkm.-Kohlm. & O.E. Erikss.	+									*J. roemerianus*	0.35
Phaeosphaeria roemeriani Kohlm., Volkm.-Kohlm. & O.E. Erikss.									+	*J. roemerianus*	0.69
Phaeosphaeria spartinicola Leuchtmann									+	*S. alterniflora*	13.8
Pleospora pelvetiae G.K. Sutherl.									+	Emergent (salt marsh)	0.35
Sphaerulina orae-maris Linder									+	Wood	0.35
Torpedospora radiata Meyers									+	Emergent	0.35
Anamorphic Ascomycetes											
Periconia sp. Tode									+	Emergent (salt marsh)	0.35
Trichocladium achrasporum (Meyers & R.T. Moore) M. Dixon ex Shearer & J.L. Crane	+									Wood	0.69

Table 4 (continued).

Species	Site		Detrital substrate	Relative frequency in all collections (%)
	1	2345678EB		
Varicosporina prolifera Nakagiri		+	*Sargassum*	0.69
Varicosporina ramulosa Meyers & Kohlm.	+	++++++++	Wood	13.9
Zalerion maritima (Linder) Anastasiou		+	Wood	0.35
Protistan slime mold *Plasmodiophora diplantherae* (Ferdinandsen & Winge) Ivimey-Cook		+	*H. wrightii*	-

Many of the fungal Orders represented in this study were the same as those of sequenced isolates obtained from deep sea hydrothermal vents (Burgaud et al. 2009) e.g., Sordariales, Xylariales, Hypocreales, Capnodiales, Pleosporales, Eurotiales, indicating members of these lineages have adapted to life in a variety of marine environments, and may point to the unexplored fate of intertidal detritus from barrier island beaches, some of which may be transported to the deep sea (Gooday et al. 1990).

Additional species isolated from direct plating of seafoam and identified by ITS rDNA sequencing were most likely of terrestrial origin and are discussed briefly here: *Cochliobolus hawaiiensis*: This plurivorous sexual ascomycete is a widespread, frequently noted non-severe plant pathogen of seeds and seedlings of rice, maize, sorghum, sugarcane and pearl millet (Wahid et al. 1988). The following asexual ascomycetes were also recorded: *Acremonium alternatum,* a hyaline hyphomycete and a saprobic, opportunistic mammalian pathogen, as well as plant endophyte (fungus occurring inside asymptomatic plant tissue) and entomopathogen (Vega 2008). *Alternaria tenuissima* is a common Pleosporalean plant pathogen and is the causal agent of leaf spot of eggplant. Its spores have a worldwide distribution. The unknown species of *Cladosporium* collected belong to one of the most common fungal genera worldwide, containing ubiquitous indoor and outdoor molds. Rarely pathogenic to humans, wind-dispersed airborne *Cladosporium* spores are found in abundance globally and can contribute to allergies and "hot tub lung". One species, *Cladosporium carrionii*, is a causal agent of chromoblastomycosis in subtropical and tropical regions, thriving at 35-37°C. An unidentified marine-derived species of *Cladosporium* produced antibiotic and antifouling compounds in culture, which inhibited attachment of bryozoan larvae and

were effective against six bacterial species (Xiong et al. 2009). *Paecilomyces variotii*, a hyaline hyphomycete, is a ubiquitous species in air and food, but it is also associated with many types of human infections and is among the emerging causative agents of opportunistic mycoses in immunocompromised hosts, causing hyalohyphomycosis (Houbraken et al. 2010).

No basidiomycete fungi were detected morphologically during this study, either through direct microscopy of substrates or dilution plating of sand and seafoam. As both white- and brown-rotting basidiomycetes decompose wood best at low pHs (Humar et al. 2001), the pH range in intertidal marine environments (7.80-9.09 in the current study) may favor ascomycete decomposition of woody detritus. Additionally, the plating methods employed during this study were selective for marine ascomycetes (non-nutritive full-salinity agar) and saltwater is known to be detrimental to the spore dispersal of most basidiomycetes.

Different ascomycete species were recovered via direct plating of seafoam and via direct staining and microscopy of seafoam. Both methods should be employed to fully capture the diversity of fungal spores trapped in seafoam. In this study, seafoam did not act as a full repository of spores for all species in the intertidal fungal community, however many species were represented by ascospores or conidia in seafoam. Additionally many featureless conidia that could not be identified morphologically were observed in seafoam during this study, indicating the need for molecular studies of intertidal seafoam.

Sand was dominated by *Corollospora maritima* in this study. *Corollospora maritima* was noted to colonize sand grains atop other substrates such as shells, worm

tubes and *Sargassum*, with the ascomata developing on the sand grains and hyphae extending into the other substrates and connecting them to the sand grains. *Corollospora* species are known to have tough, carbonaceous ascomata that are resistant to dessication and perhaps attachment to sand grains allows the persistence and ubiquity of *Corollospora* species in the highly dynamic intertidal zone of GOM barrier island beaches. Perhaps due to the visible lack of organic matter occurring in the sand at the sites sampled, arenicolous fungal diversity was lower than expected, when compared with studies of other sandy beaches in Mexico, Cuba, Texas, Japan and elsewhere (Koehn 1979, Koehn 1982, Nakagiri and Tokura 1987, González et al. 1998, González et al. 2001, González et al. 2003, Salvo and Fabiano 2007). *Varicosporina ramulosa* is the anamorph of an unknown *Corollospora* species and forms sclerocarps (sterile reproductive structures), similar to ascomata of *Corollospora* spp., to aid in its persistence in the intertidal zone (Kohlmeyer and Charles 1981). During laboratory incubation, unidentified hyphomycetes and bacteria were observed to extensively colonize the damp sand after approximately three months' incubation for all sand collections.

Emergent plant detritus (salt marsh) had high fungal diversity as detected morphologically as it has a high lignocellulose content. Intertidal wood also contained a diversity of marine ascomycete species. The following ascomycetes previously reported from mangrove wood were isolated from ascomata on detrital wood: *Acrocordiopsis patilii* at Bahia Honda FL, and *Haiyanga salina* and *Leptosphaeria avicenniae* at Caladesi Island FL. The anamorphic ascomycete *Varicosporina ramulosa* (anamorphic *Corollospora* sp.) was common on wood GOM-wide. *Corollospora maritima* and

Varicosporina ramulosa were the most commonly encountered ascomycete species on solid substrates as detected morphologically, present at all sites in both seasons, on the widest variety of substrates.

Marine plant detritus (seagrass, algae) housed fewer marine ascomycetes than emergent detritus or wood, as assessed morphologically, and this substrate type was dominated by species of *Corollospora* and *Varicosporium*. Bacteria may play more of a role in degrading this substrate type, which was heavily decomposed after 3 months' laboratory incubation. The seagrass parasite *Plasmodiophora diplantherae* was noted at Cayo Costa FL infecting detrital *Halodule wrightii*, a new location record for the GOM. See Walker and Campbell (2009) for more information and previous records of this detrimental parasite.

During laboratory incubation of substrates for 3-12 months, fungal succession of sexual ascomycete species was not observed. This is in contrast to results from studies employing sterile wood baiting techniques in marine environments, which have documented fungal succession, but over longer timescales and with fewer species documented than from naturally-occurring substrates (Jones and Hyde 1988, Alias and Jones 2000). Higher plant-based substrate diversity resulted in higher intertidal ascomycete diversity for this study, and species present at the time of collection were also detected at the end of laboratory incubations for all incubated substrates (sand, emergent, marine, wood).

Conclusions

1. Thirty-seven species of marine ascomycetes were documented morphologically during the present study. This first inventory of U.S. GOM marine fungi has increased

the number of ascomycete species reported from the U.S. GOM by over 60% and resulted in 8 new family records for the GOM. In Class Sordariomycetes: Cainiaceae, Magnaporthaceae, Xylariaceae and in Class Dothideomycetes: Massarinaceae, Mycosphaerellaceae, Phaeosphaeriaceae, Planistromellaceae and Pleosporaceae are reported here for the first time from the U.S. GOM. Certain species (*C. maritima*, *V. ramulosa*) were found GOM-wide, at all sites sampled and on a variety of substrates. Most other species are known only from a single collection.

2. High lignocellulose-containing substrates (wood, emergent plant detritus) had the highest ascomycete species richness, as detected morphologically. Sand had low diversity and was dominated by *Corollospora maritima*. Ascospores of *Corollospora* species were the most frequently collected identifiable ascospores recoverable from seafoam, and at least one may represent a new species; more material is needed to pursue this. Two other potential new *Corollospora* species were recovered from wood and detrital *Sargassum* and a green alga, respectively. At least 30% of the ascomycete diversity encountered during this study was unidentifiable, due to lack of material (e.g., a single ascospore recovered from seafoam, empty ascomata present in intertidal substrates), difficulty culturing, or no matches in the currently available literature. Next generation gene sequencing would be an ideal tool to aid in future characterizations of this diversity. Salt marsh detritus had the highest species richness of the substrates inventoried, and 70 samples may provide a representative ecological sample of this substrate. For marine plant detritus, 30 samples may suffice. More than 100 samples may be necessary to fully characterize detrital wood. Pooled seafoam and sand samples may suffice as fewer fungal taxa were recovered from these substrates.

3. Fungal succession of ascomycete species was not observed during 3-12 month substrate incubation, indicating the mycota facilitating the decay of intertidal sand beach substrates may be stable over time.

CHAPTER III

LATITUDINAL, SEASONAL AND SUBSTRATE DISTRIBUTION PATTERNS OF U.S. GULF OF MEXICO INTERTIDAL MARINE ASCOMYCETES

Introduction

Higher genetic diversity can contribute to increased productivity and resilience across multiple levels of biological organization in a range of ecosystems (Hättenschwiler et al. 2005, Balvanera et al. 2006, Hughes et al. 2008, Cardinale et al. 2011). Thus it is important to examine both the causes and consequences of intertidal detrital fungal community biodiversity. Alpha diversity is the species richness of a particular study site or ecosystem (Whittaker 1972). Beta diversity is the variation in species composition among sites in a geographic region, and is a key concept for understanding the functioning of ecosystems, the conservation of biodiversity, and ecosystem management (Legendre 2008). Gamma diversity is the overall species richness of a large region, i.e. *geographic-scale diversity* (Hunter 2002). Knowledge of all three of these diversity measures for marine fungal communities of the U.S. Gulf of Mexico will provide valuable information on species distribution and community composition in this large, mycologically understudied region.

Although latitude is known to influence the biogeography of plants and animals (Rosenzweig 1995), similar information for most fungi is lacking (Arnolds 1997), despite the important role of fungi in many ecosystems. Fungal biomass production at an ecosystem scale varies among systems and sites but can surpass 100 g C $m^{-2}year^{-1}$ (Gessner et al. 2007). Comparisons of fungal and bacterial production generally yield similar results, however, fungal biomass typically outweighs bacterial biomass and can

comprise up to 10% of total plant detrital mass in aquatic systems (Gessner et al. 2007). Such knowledge is important for understanding the structure and function of aquatic ecosystems (Gessner et al. 2004, Morin and Steed 2004) and can provide critical insights for habitat monitoring, conservation and restoration activities.

In recent years, intertidal beach fungi have been inventoried in studies of one or two beaches in Egypt (Migahed 2003), Portugal (Figueria and Barata 2007, Azevedo et al. 2012), the South Baltic Sea (Mudryk and Podgórska 2007), Malaysia and Singapore (Sundari et al. 1996), and Italy (Onofri et al. 2011). However, no thorough regional inventory has ever been conducted, and basic diversity and distribution data are lacking for this ecologically important group of organisms. To characterize ascomycete diversity and distribution patterns in the U.S. GOM, fungi were inventoried along latitudinal gradients at selected barrier island sites in Texas and Florida. Species occurrence and relative abundance data were used to assess the roles of 1) latitude (warm-temperate vs. subtropical); 2) season (summer vs. winter); 3) substrate type (wood, marine plant detritus, emergent plant detritus); and 4) environmental factors (salinity, water temperature) in structuring the ascomycete communities found on drift intertidal substrates.

Materials and Methods

For information on intertidal substrate collection and fungal isolation methods, see Chapter II.

Molecular Fingerprinting of Intertidal Ascomycete Communities via ITS T-RFLP Analysis

As the morphological inventory (Chapter II) only identified fungal taxa reproducing at the time of collection, molecular community fingerprinting was also conducted. Molecular characterization of marine ascomycete communities associated with intertidal drift substrates (wood, marine plant detritus [seagrass and algae], emergent plant detritus [salt marsh and mangrove]) was conducted using Terminal Restriction Fragment Length Polymorphism (T-RFLP) analysis of the internal transcribed spacer (ITS) region of fungal ribosomal DNA. T-RFLP has been used successfully in the characterization of salt marsh plant marine fungal communities (Buchan et al. 2002, Walker and Campbell 2010) and is a DNA fingerprinting technique that separates PCR-generated DNA products from environmental samples. PCR of environmental DNA can generate templates of differing DNA sequences that represent many of the microbial organisms present. By using fungal-specific PCR primers, DNA of the fungal community is isolated and then PCR products are separated based on DNA sequence differences due to the differing fragment lengths produced by restriction enzyme digestion. The T-RFLP technique was also chosen to allow comparison with the reference T-RF size databases generated by two prior molecular studies of marine saprotrophic ascomycetes (Buchan et al. 2002, Walker and Campbell 2010). The *Hae*III restriction enzyme was chosen for several reasons. First, Clement et al. (1998) found it yielded the highest number of terminal restriction fragments (TR-Fs) when compared with three other restriction enzymes. Buchan et al. (2002) found *Hae*III best discriminates between marine ascomycete species, as the size of the T-RF produced is conserved within species yet is sufficiently variable to discriminate between species. The recognition site for *Hae*III is the DNA nucleotide sequence GGCC; the restriction enzyme cleaves between the second

and third nucleotide (G/C), generating multiple restriction fragments for each fungal species. Only the terminal restriction fragment is fluorescently labeled and its size is detected by capillary electrophoresis. Thus, each T-RF is considered to represent a different fungal species and the number of T-RFs in a fungal community profile can be used as a conservative proxy for species richness (Avis et al. 2006).

Community DNA Extraction from Intertidal Substrates

DNA was extracted from intertidal solid substrate samples in triplicate using an UltraClean Soil DNA Kit with vortex adaptor (MoBio). The fungal ITS rDNA region was amplified using the ascomycete-specific primers ITS 1-F: 5’ CTT GGT CAT TTA GAG GAA GTA A 3’ (Gardes and Bruns 1993) and ITS 4-A: 5’ CGC CGT TAC TGG GGC AAT CCC TG 3’ (Larena et al. 1999) (Invitrogen), with ITS 1-F labeled on the 5' end with the fluorescent dye FAM (6-carboxyfluorescein) following the thermocycling parameters mentioned above. Target specificity was determined by agarose gel electrophoresis and the reaction yield was quantified using a NanoDrop ND-1000 spectrophotometer. PCR products were cleaned using a QIAquick PCR purification kit (Qiagen), followed by restriction digestion using the *Hae*III restriction enzyme. For each sample, a restriction digest reaction was set up in a 1.5 ml microcentrifuge tube containing either 10 ng (isolate) or 100 ng (community) purified PCR product, 1 μl SuRE/Cut buffer M (10X), 1 □l (10 U) *Hae*III enzyme and ddH_2O to equal a 10 μl total reaction volume. This reaction was incubated at 37°C for 3 h using a dry bath incubator. Following digestion, the digested PCR product was stored at -20°C until analysis. All restriction digest reagents were obtained from Roche Applied Science and were stored at -20°C.

Individual peaks were further characterized by subjecting individual fungal species in culture to the T-RFLP technique to obtain the T-RF size for each species, allowing further interpretation of community profiles (Figure 12). DNA was extracted from single spore isolates using a Qiagen DNeasy Plant Mini Kit with the addition of lyticase (Raja et al. 2003). The non-coding internal transcribed spacer (ITS) region of fungal ribosomal DNA was targeted for polymerase chain reaction (PCR) amplification due to its highly conserved nature and high sequence variability at the species level (Larena et al. 1999). DNA was amplified using fungal ITS primers ITS 1-F: 5' CTT GGT CAT TTA GAG GAA GTA A 3' (Gardes and Bruns 1993) and ITS 4-A: 5' CGC CGT TAC TGG GGC AAT CCC TG 3' (Larena et al. 1999) (Invitrogen) with ITS 1-F labeled on the 5' end with the fluorescent dye FAM (6-carboxyfluorescein). PCR was performed using the following thermocycler parameters: a 3 min initial denaturation step at 95°C, followed by 35 cycles of 1 min at 95°C, 30 s annealing at 52°C and 1 min extension at 72°C, ending with a 10 min final extension at 72°C in a Thermo Electron Px2 thermal cycler. PCR products were purified using a QIAquick PCR purification kit (Qiagen).

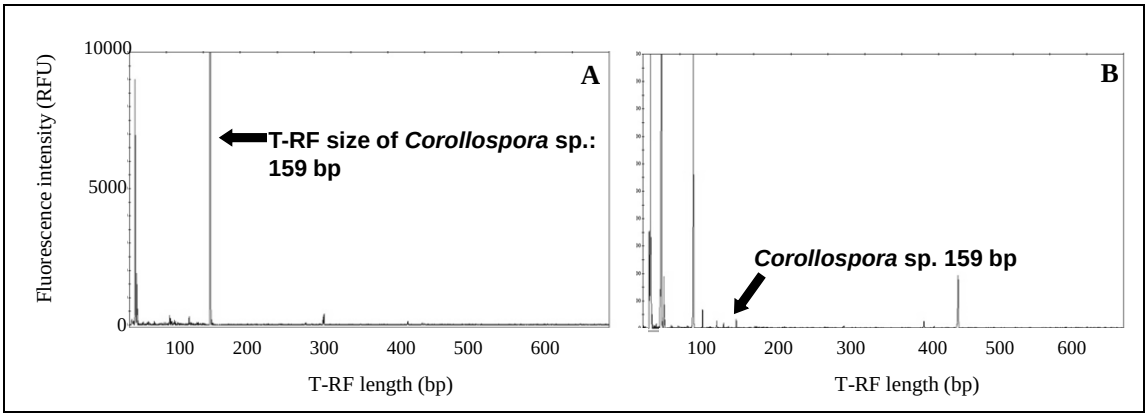

Figure 12. Matching single species isolates to community fingerprints using ITS T-RFLP analysis. A: T-RF size of *Corollospora* sp. in pure culture (159 bp). B. ITS T-RFLP community fingerprint from detrital *Halodule wrightii* collected from Caladesi Island, Florida (5 ascomycete species, including *Corollospora* sp.).

Samples were subjected to the T-RFLP analysis technique as mentioned below for environmental samples. All T-RFLP samples were analyzed at the University of Illinois Urbana-Champaign Core Sequencing Facility using an Applied Biosystems 3730*xl* DNA Analyzer. Each fluorescently-labeled PCR reaction was processed in triplicate and the resulting three chromatograms were overlaid for each sample using GeneMapper™ Version 3.7 software. Peaks were standardized using the R-based program PAST (2012). Peak height in T-RFLP chromatograms was used as a proxy for the relative abundance of fungal taxa represented by restriction fragments (Stepanauskas et al. 2003). Peaks were assumed to be artifacts and removed from analysis if they did not contribute more than 1% to the sum of all peak heights in any individual profile and occurred in less than three profiles (Stepanauskas et al. 2003). Additionally, peaks smaller than 50 bp or larger than 550 bp were assumed to be primer and uncut ITS sequences, respectively, and were excluded from the analysis.

Limitations of T-RFLP Analysis

It should be noted that the high number of ascomycete species recovered in the T-RFLP analysis includes ascomycete yeasts, which were not inventoried morphologically. T-RFLP data can be used for relative quantification and statistical analysis, although DNA sequence data cannot be definitively inferred directly from the T-RFLP profile (Abdo et al. 2006). In cases where more than one peak was observed for the same T-RF, the first peak was used to obtain a representative T-RF size as the second peak was most likely the product of an incomplete restriction digest (Clement et al. 1998). For additional caveats of ascomycete ITS T-RFLP analysis, see Walker and Campbell (2010). T-RFLP analysis can detect the presence of a species, but not the absence of one (Dickie

et al. 2002). Correlating T-RF size with species by morphological identification as done in this study helps to avoid biases introduced by sampling methods, fungal genomic structure, and restriction enzyme characteristics (Avis et al. 2006).

Statistical Analysis

Beta diversity (β) was calculated using the formula proposed by Wilson and Shmida (1984), the most commonly used method for measuring the continuity of species between communities (Koleff et al. 2003). To complement the β diversity analysis, fungal similarity among different sites was calculated using Sørenson's similarity index (Sørenson 1948): Sørenson's similarity index = 2c/(a + b); where a = total number of species in the first community; b = total number of species in the second community; and c = number of species both communities have in common.

To perform multivariate statistical analysis on the T-RFLP data, the data were first converted to a "sample by taxa table" which depicts the different samples (T-RFLP profiles) versus the individual taxa (T-RFs), with peak area as species relative abundance values. Species richness (S), Pielou's evenness (J') and Shannon diversity (H'[$\log_e$]) were calculated for each site using the DIVERSE module of Plymouth Routines in Multivariate Ecological Research software (PRIMER v.6.1.6, PRIMER-E Ltd, Plymouth, UK)(Clark and Warwick 2001). All statistical tests were performed using PRIMER v6.1.6 software (Clarke and Gorley 2006) unless otherwise noted. T-RF relative species abundances were log (x+1) transformed to compress the scale of comparison prior to calculation of Bray-Curtis similarity matrices (Bray and Curtis 1957). The effect of latitude on fungal community composition was visualized by CLUSTER analysis using the Group Average algorithm to produce the dendrogram. The similarity percentages

(SIMPER) routine was applied to decipher percentage contributions from each T-RF (i.e., each species) to the similarity and dissimilarity of each sample in relation to the others. Two-dimensional non-metric multidimensional scaling (MDS) was used to visualize ascomycete community similarity, with communities with similar relative species abundances placed closer together in ordination space (Clarke 1993). A Shepard plot of obtained versus observed ranks was produced to indicate the quality of the MDS plot.

Analysis of Similarity (ANOSIM) tests were conducted to assess the roles of latitude, season and substrate in structuring fungal communities. ANOSIM is a multivariate randomization test analogous to ANOVA performed on a similarity matrix and producing a test statistic, R, which assesses the null hypothesis of no among-group differences. $R \sim 0$ when there are no significant differences among groups; greater among-group differences are indicated as R approaches -1 or 1. The significance of the R statistic is calculated from randomization tests on the similarity matrix (Clark and Warwick 2001). A two-way crossed ANOSIM with replicates was used to assess the role of season (2 levels: winter and summer) across substrate (5 levels: sand, seafoam, marine, emergent, wood) on the data after it was standardized to sampling effort. The BEST procedure was used to find matches between the among-sample patterns of the TRF communities, and any patterns from the abiotic variables associated with those samples, using the Spearman rank coefficient and an Euclidean distance resemblance measure with 999 permutations.

Canonical Correspondence Analysis (CCA) (Legendre and Legendre 1998) was also employed to test if variation in community composition was explained by water temperature or salinity. In CCA, the ordination axes are linear combinations of the

environmental variables. CCA is an example of direct gradient analysis, where the gradient in environmental variables is known *a priori* and species abundances (or presence/absences) are considered to be a response to this gradient. The eigenanalysis algorithm given in Legendre and Legendre (1998) was used, with ordinations given as site scores. Environmental variables were plotted as correlations with site scores. Canonical analysis has been used recently for analysis of aquatic mangrove fungal communities (Schmit and Shearer 2004) and Ingoldian mitosporic ascomycete communities (Nikolcheva and Bärlocher 2005). CCA was performed using the PAST v.2.15 program for T-RFLP data analysis in R v.2.14.1 software.

Results

Twenty-eight ITS T-RFLP ascomycete community profiles were generated during the course of this study to assess ascomycete species richness and relative abundance. Gamma diversity of the study region (TX and FL combined) was 131 species, with 49% of T-RFs (species) occurring as singletons. Individual samples housed from seven to 31 T-RFs. The most common T-RF sizes were: 58 bp, in 93% of the profiles (matched to *Corollospora maritima* using single spore isolate T-RF analysis, this was also the most commonly encountered species in the morphological inventory (Table 4); 71 bp in 12% of the profiles (matched to common salt marsh ascomycete *Phaeosphaeria spartinicola*) and 159 bp occurring in 6% of the profiles (matched to an undescribed *Corollospora* species)(Figure 9). Species richness (S), evenness (J') and Shannon diversity (H'[log $_e$]) for each site across all substrate types are given in Table 5, using the DIVERSE analysis on an aggregated data matrix in PRIMER v.6.1.6.

Table 5

Species richness (S), Pielou's evenness (J') and Shannon diversity (H'[log $_e$])by site, as calculated from T-RF species richness and relative abundance data

Site	S	J'	H'(log $_e$)
1. South Padre Island	57	0.728985	2.947323
2. Mustang Island	61	0.733071	3.013564
3. Galveston Island	46	0.769617	2.946589
5. St. Vincent Island	7	0.769133	1.496663
6. Caladesi Island	25	0.796515	2.563881
7. Cayo Costa Island	11	0.687413	1.648345
8. Bahia Honda	46	0.72789	2.786829

When compared with results of the morphological inventory in Chapter II (Figure11, p. 40), far more ascomycete species were detected per sample using molecular fingerprinting than by morphology alone (Figure 13).

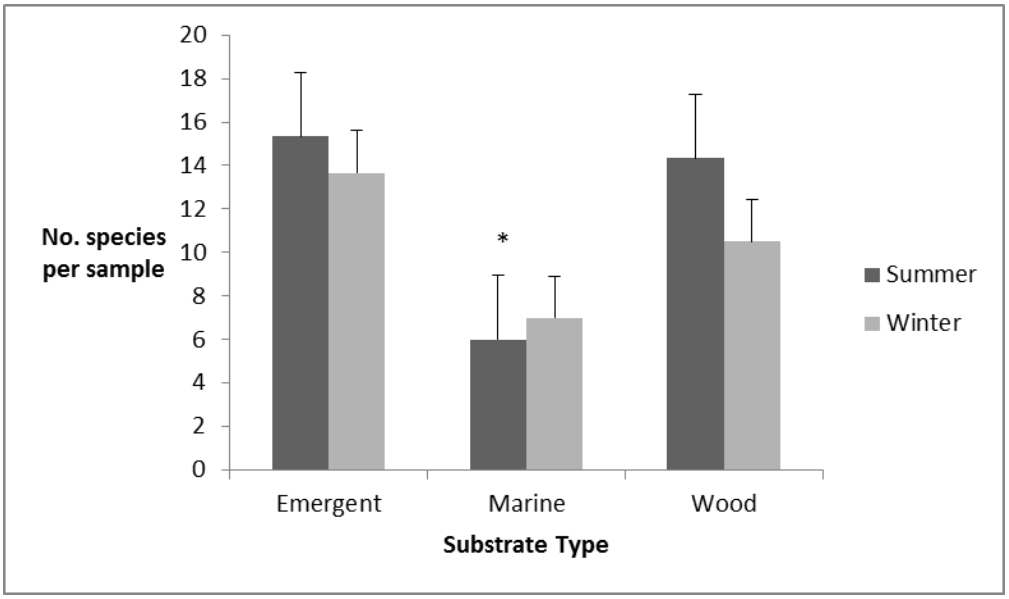

Figure 13. ITS T-RF species richness by substrate type and season (standardized to sampling effort). T-RFs were used as proxies for individual ascomycete species. Significant differences in species richness by substrate type are denoted by an asterisk (*). Error bars denote standard error.

Substrate

Morphological and molecular data indicated different ascomycete communities were present on different substrate types, with different fungal taxa dominating each substrate type. The highest species richness was encountered on substrates rich in lignocellulose (wood, emergent plant detritus) (Figure 13). Unfortunately, due to fewer substrates collected and difficulty in DNA amplification, molecular community data was only collected for one substrate at St Vincent Island FL (summer *Sargassum*) and it had < 30% species in common with Cayo Costa FL and Caladesi Island FL marine substrates. All pairs of substrates showed significant differences ($P < 0.05$) in species composition with exception of the substrate pair Emergent/Wood ($P > 0.1$)(Table 6).

Table 6

Global R statistic and pairwise comparisons of fungal community similarities between different substrates based on T-RF species relative abundance data. R statistics and P values from ANOSIM

Global R statistic	Significance	Number of observations	
0.247	$P < 0.02$	28	
Pair of substrates	R statistic	Significance	Number of observations
Marine/Emergent	0.335	$P < 0.02$	11
Marine/Wood	0.226	$P < 0.05$	9
Emergent/Wood	-0.075	$P > 0.1$	337

Results of a two-way crossed ANOSIM with replicates indicate no significant seasonal difference across substrate types (R=0.076, $P>0.1$). However, a significant difference in species richness by substrate was noted for the substrate pairs Marine/Emergent (R=0.541, $P<0.003$) and Marine/Wood (R=0.426, $P<0.05$) across seasons (Figure 13).

Latitude

Latitudinal differences in the marine fungal communities colonizing the same substrate type were observed. Species richness pooled across all substrate types increased with decreasing latitude in both TX and FL. Higher ascomycete species richness was noted on detrital seagrass (*Halodule wrightii*) at lower latitudes in FL (Caladesi Island, Cayo Costa Island) than in the FL panhandle (St. Vincent Island).

From both morphological and molecular data obtained during this dissertation research, alpha diversity (ascomycete species richness)(Table 5) was highest at South Padre Island and Mustang Island, TX and Bahia Honda, FL, the southernmost sites sampled. Beta diversity (amount of ascomycete species change between sites) was highest between Galveston Island, TX and Caladesi Island, FL and lowest between St Vincent Island, FL and Cayo Costa, FL (Table 7). Surprisingly, the most similar sites based on Sørenson's Index were St Vincent Island, FL and Cayo Costa, FL (44% similarity), and St Vincent Island, FL and Mustang Island, TX (41% similarity). However, the southernmost sites in both TX and FL were similar with one another (Mustang Island and South Padre Island {38%}; Cayo Costa and Bahia Honda, FL {38%}).

For Texas samples, cluster and MDS visualization revealed fungal communities from South Padre Island and Mustang Island intertidal substrates clustered together while most fungal communities from Galveston Island were distinct (Figures 14, 15).

Table 7
Sørenson's similarity index and index of beta diversity (Wilson and Schmida 1984) among 3 Texas and 4 Florida collection sites based on ITS T-RFLP data. 1=South Padre Island, TX; 2=Mustang Island, TX; 3=Galveston Island, TX; 5=St. Vincent Island, FL; 6=Caladesi Island, FL; 7=Cayo Costa Island, FL; 8=Bahia Honda, FL. For site locations see Figure 4

	Texas			Florida			
SITES	1	2	3	5	6	7	8
1	-	0.38/0.63	0.18/0.82	0.29/0.71	0.26/0.74	0.25/0.75	0.23/0.77
2		-	0.16/0.84	0.41/0.59	0.27/0.73	0.37/0.63	0.35/0.66
3			-	0.13/0.87	0.05/0.95	0.11/0.89	0.22/0.78
5				-	0.32/0.68	0.44/0.56	0.16/0.84
6					-	0.36/0.66	0.16/0.84
7						-	0.38/0.76
8							-

Winter *Thalassia testudinum* fungal communities clustered together from South Padre Island and Mustang Island. For Florida samples, Bahia Honda samples grouped together (Figure 14), with summer marine (*Sargassum* sp.) and winter marine (*Syringodium filiforme*) showing the most similarity amongst FL marine fungal communities, followed by summer red mangrove fruit and summer wood. MDS visualization revealed an overlap between South Padre Island and Mustang Island communities (Figure 15).

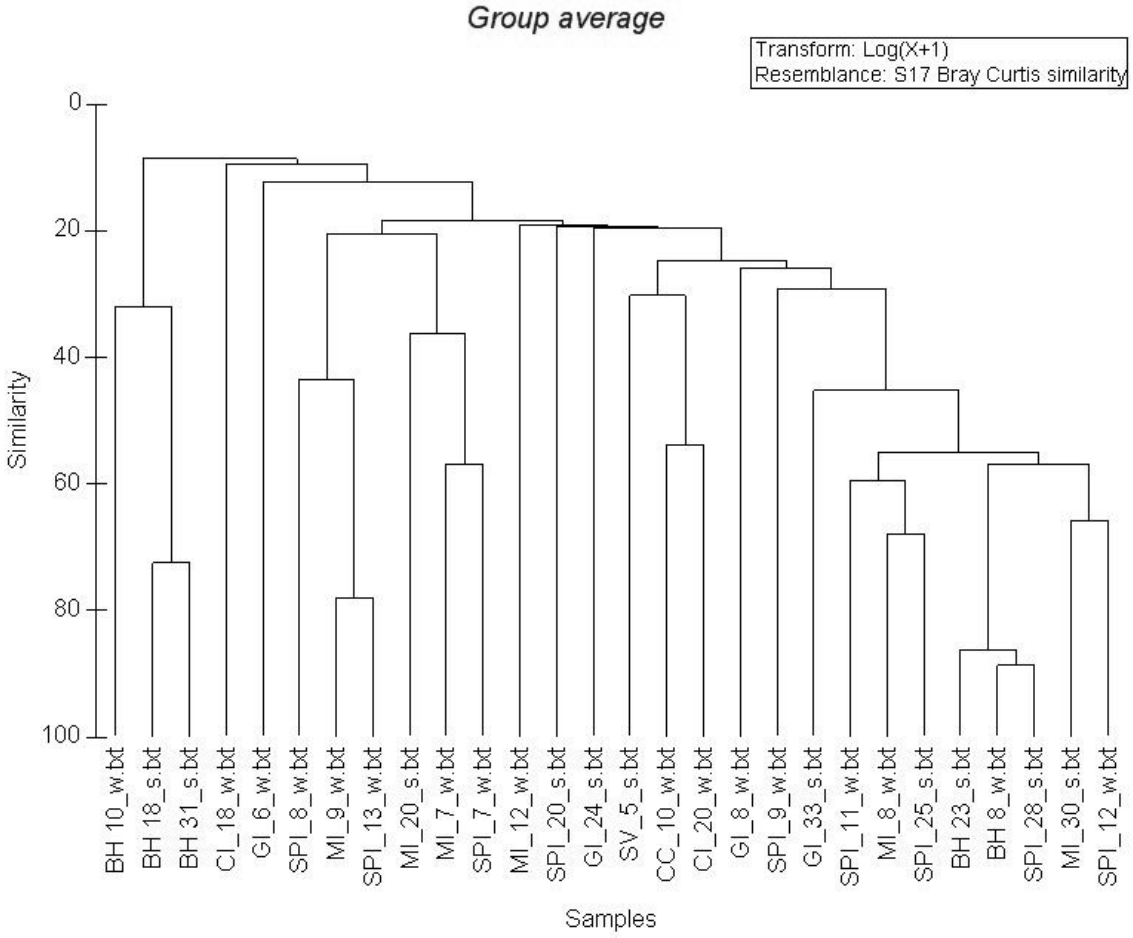

Figure 14. Gulf of Mexico ITS T-RF relative species abundance data visualized by cluster analysis using the Group Average algorithm on a Bray-Curtis similarity matrix. Sample names ending in ‘w’ were collected in winter; samples ending in ‘s’ were collected in summer.

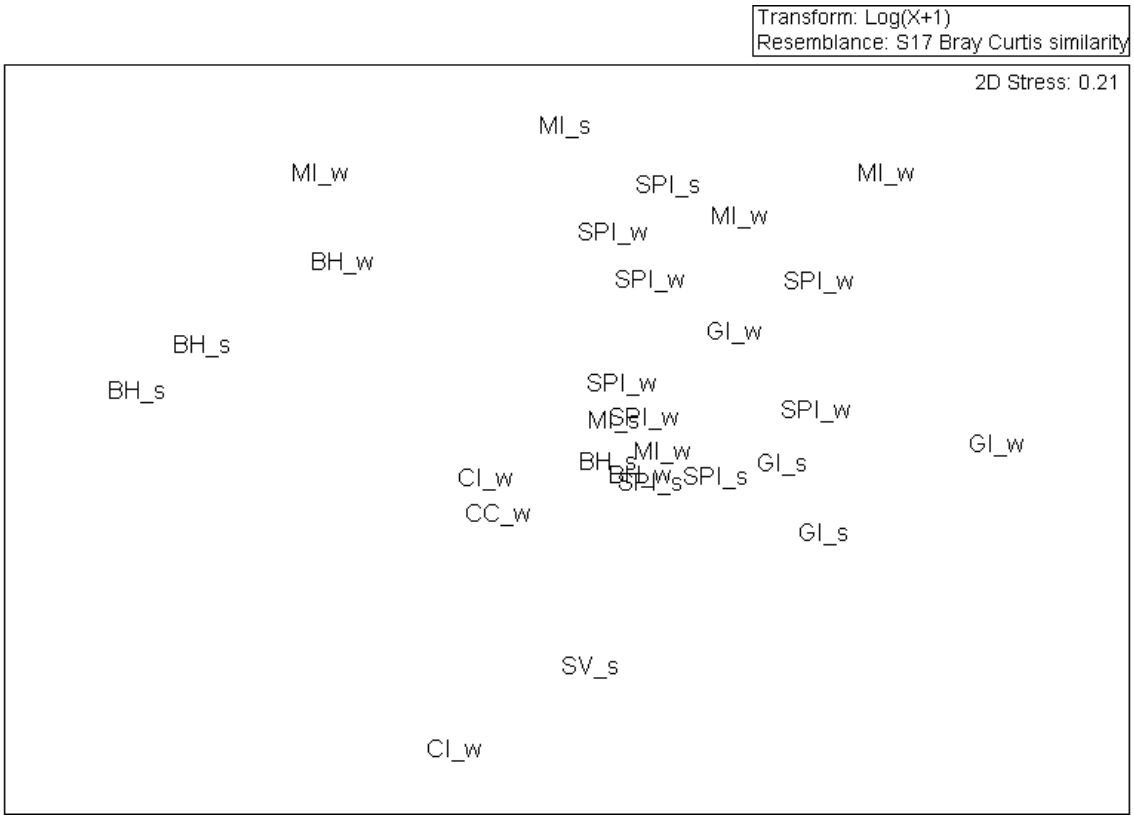

Figure 15. Gulf of Mexico ITS T-RF relative species abundance data visualized by 2D MDS ordination (Bray-Curtis similarity matrix). BH = Bahia Honda, CC = Cayo Costa, CI = Caladesi Island, SV = St. Vincent Island, GI = Galveston Island, MI = Mustang Island, SPI = South Padre Island. Sample names ending in 'w' were collected in winter; samples ending in 's' were collected in summer.

Little overlap was found between TX and FL communities when visualized by MDS, other than for the southernmost sites (South Padre Island TX and Bahia Honda FL). A Shepard diagram (Figure 16) reveals how the stress of the MDS was calculated.

Three detrital marine plant samples (one from South Padre Island and two from Bahia Honda) had 85% community similarity (Figure 14). Three additional samples grouped together from Bahia Honda: mangrove fruit and mangrove wood had 50% community similarity, and a *Sargassum* sample had 30% community similarity with both mangrove fruit and mangrove wood. In Texas, a saltmarsh sample from South

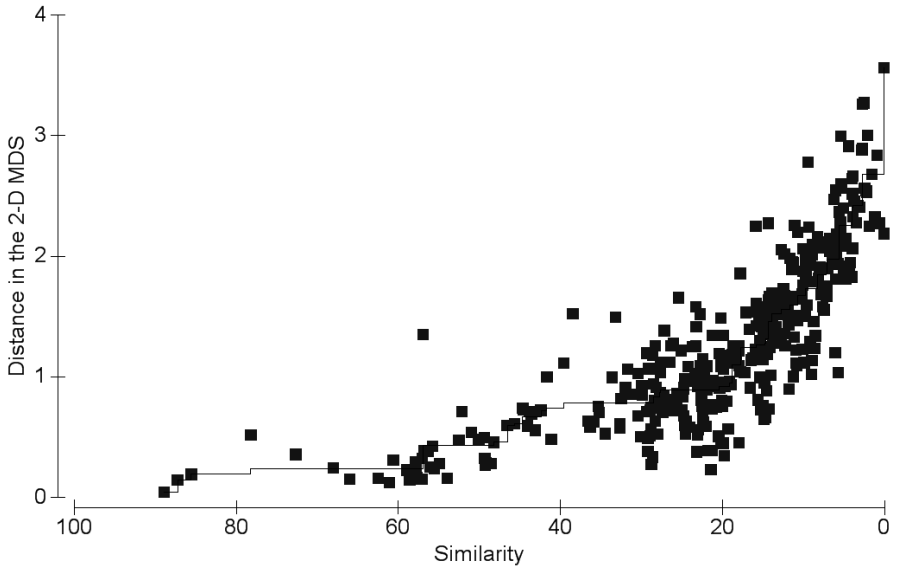

Figure 16. Shepard diagram for 2D MDS of ITS T-RF relative species abundance data. This scatter plot represents the pairwise distances between samples in the final ordination against the dissimilarities in the Bray-Curtis resemblance matrix. The stress of the MDS measures the departure of points (squares) from the regression line-of-best-fit.

Padre Island and a wood sample from Mustang Island had 75% community similarity, indicating overlap in fungal communities between high lignocellulose-content substrate types. Saltmarsh samples taken from Galveston Island in summer and winter had only 10% similarity and did not cluster near any of the other communities, indicating the high fungal diversity in saltmarsh detritus. Similarly, a saltmarsh sample from Caladesi Island, FL had less than 10% similarity with any other community sampled during this study (Figure 14, 15). SIMPER analysis revealed the ascomycete species represented by the T-RF length 50 bp provided 23.59% of the dissimilarity between clusters on the dendrogram. Twelve other species contributed 8.42% or less to the discrimination between groups.

For site comparisons, significant differences ($P < 0.05$) in fungal communities were noted between the following site pairs: BH/CI, BH/GI, BH/SPI, and GI/SPI using analysis of similarities (ANOSIM) (Table 8).

Table 8

Pairwise comparisons of fungal community similarities between different sites based on T-RF species relative abundance data for all substrates. R statistics and P values from ANOSIM

	Global R statistic	Significance	Number of observations
	0.322	$P < 0.004$	28
Pair of sites	R statistic	Significance	Number of observations
BH, CC	0.4	$P > 0.1$	1
BH, CI	0.491	$P < 0.05$	1
BH, GI	0.494	$P < 0.04$	5
BH, MI	0.251	$P < 0.07$	32
BH, SPI	0.495	$P < 0.004$	3
BH, SV	0.4	$P > 0.1$	1
CC, CI	0	$P > 0.1$	2
CC, GI	0.25	$P > 0.1$	2
CC, MI	-0.067	$P > 0.1$	5
CC, SPI	0.395	$P > 0.1$	3
CI, GI	0.679	$P < 0.07$	1
CI, MI	0.229	$P > 0.1$	6
CI, SPI	0.637	$P < 0.06$	3
CI, SV	0	$P > 0.1$	2
GI, MI	0.095	$P > 0.1$	50
GI, SPI	0.343	$P < 0.05$	32
GI, SV	0.833	$P > 0.1$	1

Table 8 (continued).

MI, SPI	0.028	$P > 0.1$	347
MI, SV	0.356	$P > 0.1$	3
SPI, SV	0.66	$P < 0.1$	1

Season

Cluster and MDS analyses revealed a slight seasonal effect at the TX sites: fungal communities on intertidal wood were distinct in summer and winter from South Padre Island. Winter wood, emergent plant detritus (salt marsh) and marine plant detritus (*Halodule wrightii*) communities from South Padre Island and Mustang Island grouped together, and summer wood communities from South Padre Island and Mustang Island grouped together, but with less similarity than the winter communities. Galveston Island summer and winter detrital salt marsh communities had only 10% similarity (Figures 13,14) indicating a possible role of seasonality in saltmarsh fungal communities. The role of season was also evidenced by separate *Sargassum* fungal communities in summer and winter at Bahia Honda FL, with < 10% similarity. GOM-wide, a seasonal trend was noted for marine detritus only, with some samples exhibiting higher species richness in winter. Wood and emergent detritus had similar fungal species richness in summer and winter, while marine detritus had higher species richness in winter, with up to 31 ascomycete species present on a single sample. However, when all GOM samples were analyzed for the effect of season on community composition using a one-way ANOSIM, no significant seasonal effect on fungal communities was noted ($R = 0.03$, $P > 0.1$).

Environmental factors

Water temperatures recorded during this latitudinal study of TX and FL sites ranged from 15.4-19.0°C in winter and 26.6-30.7°C in summer. Salinities recorded ranged from 19.5-33.5 in winter and 26.6-35.0 in summer. CCA did not reveal an effect of salinity or water temperature on marine ascomycete species richness or relative species abundance for the sites sampled. The results of the BEST procedure to examine environmental variables revealed no significant correlation of water temperature, salinity, pH or season with ascomycete relative species abundance (Rho=0.234, $P>0.1$).

Discussion

Changes in marine ascomycete community composition with latitude in both Texas and Florida are similar to patterns found in other geographical locations for marine fungi (Hughes 1974, Booth and Kenkel 1986, Shearer and Burgos 1987), Ingoldian mitosporic ascomycetes (Miura 1974, Wood-Eggenschwiler and Bärlocher 1985), and freshwater ascomycetes (Raja 2007). Similar substrates collected at similar latitudes may play a role. Additionally, the high β diversity between Galveston Island TX and Caladesi Island FL could indicate a longitudinal factor involved in structuring these two intertidal communities.

Some seasonal differences in ascomycete communities were noted, although GOM-wide there was no statistically significant seasonal effect on ascomycete species richness or relative abundance on any substrate type. Epiphytic communities on seagrass leaves consist of fungi, bacteria, algae and invertebrates, and epiphyte load can increase when nutrients become elevated in the water column (Mutchler and Dunton 2007). As

nutrient levels increase through eutrophication, grazing by crustaceans, gastropods and other organisms may counteract the increased growth of epiphytes up to some threshold level when epiphyte growth overwhelms the grazing rate (Burkholder et al. 2007). A prior study conducted near Mustang Island, Texas (site 2 in the present study) indicated seagrass epiphyte load may be higher during winter, possibly due to reduced leaf elongation and reduced grazer activity during the colder months (Mutchler and Dunton 2007). This may explain the higher ascomycete species richness observed for marine plant detritus in winter GOM-wide.

The morphological inventory (Chapter II) detected distinct ascomycete communities on the separate substrate types, with some overlap of abundant generalist species such as *Corollospora maritima* and *Passeriniella obiones*. Although the molecular study indicated similar fungal species richness for wood and emergent detritus, the morphological inventory revealed more species fruiting on emergent detritus (salt marsh). This high diversity may represent functional redundancy of salt marsh fungal taxa in the presence of large quantities of lignocellulose. Marine detritus, which contains less lignocellulose, had lower species richness, as detected both molecularly and morphologically. Fungal activity and thus detrital decomposition rates are regulated by internal (e.g., detrital nutrient concentrations and carbon quality) and external (e.g., temperature, dissolved nutrient concentrations) factors (Gessner et al. 2007). As fungi grow in detritus, resources are partitioned between the vegetative mycelium and the reproductive structures with much detrital biomass ultimately leaving the intertidal zone as fungal spores. Thus, small islands of intertidal plant detritus may function as microhabitats for fungi with most fungal species adapted to certain substrates, while a

small number of generalist species are able to utilize a wide range of intertidal substrate types. Fungal community composition is known to vary even at different locations on a single plant (Gessner 1977, Van Ryckegem et al. 2007), which emphasizes the potential importance of substrate differences at different spatial scales for both fungal community composition and function. Previous studies limited to morphological techniques have found distinct saprotrophic fungal communities present on *S. alterniflora* and *J. roemerianus* (Newell and Porter 2000). Walker and Campbell (2010), combining morphological and molecular techniques, indicated the fungal taxa present on the two host plants may overlap by as much as 50%, although it should be noted there was no overlap in taxa detected morphologically.

Differences in ascomycete communities were noticed within substrate type in the current study, for example marine detritus at Mustang Island, TX. The two seagrasses and one green alga analysed had ~18 % ascomycete species overlap (8/45 species), with each plant exhibiting a different dominant ascomycete taxon based on % abundance data. The seagrass *Thalassia testudinum* housed the most diverse ascomycete community, but all three samples exhibited similar species richness (22, 18, and 18 species, respectively). Seagrass beds provide habitat for fish and invertebrates, food for waterfowl, help reduce sediment suspension and act as water quality indicators (Orth et al. 2006). Unfortunately, seagrasses worldwide are under direct threat from a variety of human influences in coastal zones, including increased nutrient and sediment runoff, introduction of invasive species, hydrological alterations and commercial fishing practices (Orth et al. 2006). Along the U.S. GOM coast, human influences include increased commercial and

recreational bay use, increased nutrient and contaminant loading, and growing populations (Pulich and Onuf 2007). Knowledge of the species identities and roles of saprotrophic, mutualistic and pathogenic fungi will aid in the conservation and restoration of these threatened ecosystems.

Water temperature is known to be a primary factor controlling seasonal growth of submerged aquatic plants such as seagrasses (Lee and Dunton 1996; Lee et al. 2007; Mutchler and Dunton 2007). Optimal growth temperature for subtropical seagrass species is between 23°C and 32°C (Lee et al. 2007). Water temperature is also thought to play a role in determining marine fungal distribution (Kohlmeyer and Kohlmeyer 1979). However CCA analysis revealed no effect of either water temperature or salinity on ascomycete species richness or relative abundance on intertidal substrates sampled in the U.S. GOM. Finally, the role of wind and aerosolized dispersal of marine fungal spores need to be examined, as both have been shown to be important mechanisms for prokaryotic microbial dispersal in coastal environments (Dueker et al. 2012). Passive spore dispersal in seawater may be only one of multiple ways marine fungi colonize intertidal substrates.

Conclusions

1. Sørenson's similarity index and β diversity comparisons among sites revealed similar marine fungal communities at similar latitudes. Species richness increased with decreasing latitude, perhaps due to increased substrate diversity at the southern collection sites. Cluster analysis and MDS revealed some grouping of intertidal fungal communities by latitude (South Padre Island and Mustang Island fungal communities in TX, Bahia Honda fungal communities in FL, and South Padre and Bahia Honda communities).

ANOSIM revealed significant ascomycete community differences between the following site pairs: BH/CI, BH/GI, BH/SPI, GI/SPI, which may indicate roles of both latitude and longitude in structuring marine ascomycete communities.

2. Seasonal differences in species richness and relative abundance were noted for marine plant detritus, possibly due to reduced grazing pressure in winter, and for saltmarsh detritus at Galveston Island. However, based on ANOSIM, no GOM-wide significant seasonal differences in ascomycete community structure were noted.

3. Cluster analysis and MDS revealed some grouping of intertidal fungal communities by substrate. ANOSIM revealed a significant difference in ascomycete communities between the detrital substrate type pair Marine/Emergent and the pair Marine/Wood. The substrate pair Emergent/Wood did not have significantly different ascomycete communities, and some overlap in ascomycete species between these two substrate types with high lignocellulose content was also noted in the morphological inventory.

4. The CCA and BEST multivariate statistical procedures did not reveal an effect of salinity, water temperature or pH on ascomycete community composition.

CHAPTER IV

THE ROLE OF SAMPLING FREQUENCY IN SPECIES RICHNESS ESTIMATES FOR MARINE FUNGI, WITH RECOMMENDATIONS FOR OPTIMAL SAMPLING OF INTERTIDAL ASCOMYCETES

Introduction

The question of how to obtain a representative ecological sample of an intertidal fungal community has not been adequately addressed in the marine mycological literature. Kohlmeyer et al. (2004) found that 20 dead *Juncus roemerianus* leaves provided a representative sample of the fungal community in their intensive North Carolina salt marsh study, with certain species being collected almost every month over two years. Other species, considered rare by the authors, were collected only once or twice during this time. Based on their experience, Kohlmeyer et al. (2004) suggest a similar sampling strategy for other saltmarsh plants. However, the sampling intensity needed to adequately characterize fungal assemblages on other substrates in other marine habitats and geographic regions is currently unknown.

The occurrence and distribution of marine ascomycetes on sand beaches is influenced by multiple factors, some of which cannot be controlled by the investigator (e.g., substrate type availability and substrate abundance), while other factors, such as sampling frequency, can be subjected to methodological optimization (Jones 2000, Azevedo et al. 2012). The objectives of this study were: 1) to characterize the marine fungal assemblages found on different intertidal substrates on two sand beaches in coastal Mississippi; 2) to compare differences and similarities among the fungal assemblages

associated with each substrate type; and 3) to examine the effect of sampling frequency on species richness estimates.

Materials and Methods

To investigate the role of sampling frequency on marine fungal species richness from intertidal GOM substrates, East Beach, Ocean Springs, MS (30°23'40" N 88°48'36" W) was sampled weekly for 4 weeks (May 2010) and West Ship Island, MS (30°12'27.52" N 88°57'49.24" W) was sampled every other month for ten months (April 2009-January 2010) (Figure 17). For transect and substrate sampling methodology see Chapter II. Intertidal substrates were examined immediately upon returning to the laboratory on the day of collection. For fungal morphological identification techniques see Chapter II. Differences in species richness among substrate types were assessed with one-way ANOSIM in PRIMER v6.1.6 (Clarke and Gorley 2006).

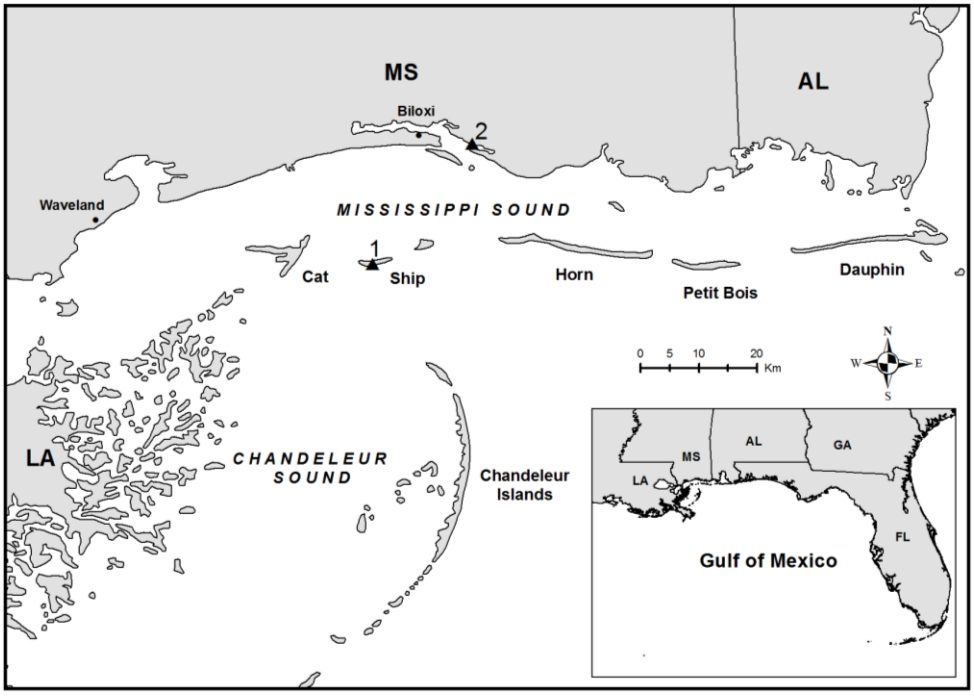

Figure 17. Intertidal sand beach collection sites in Mississippi. 1) West Ship Island. 2) East Beach (Ocean Springs).

Results

Substrate availability varied from week to week at East Beach and from month to month at West Ship Island (Table 9). Of the solid substrates collected, emergent plant detritus (salt marsh) had the highest marine fungal species richness at both sites (Figure 18). At East Beach, species richness was fairly constant (S=8-10) each week during May 2010 (Figure 19A) and at West Ship Island species richness peaked in October (S=12) during the 10 months sampled (April 2009-January 2010) (Figure 19B).

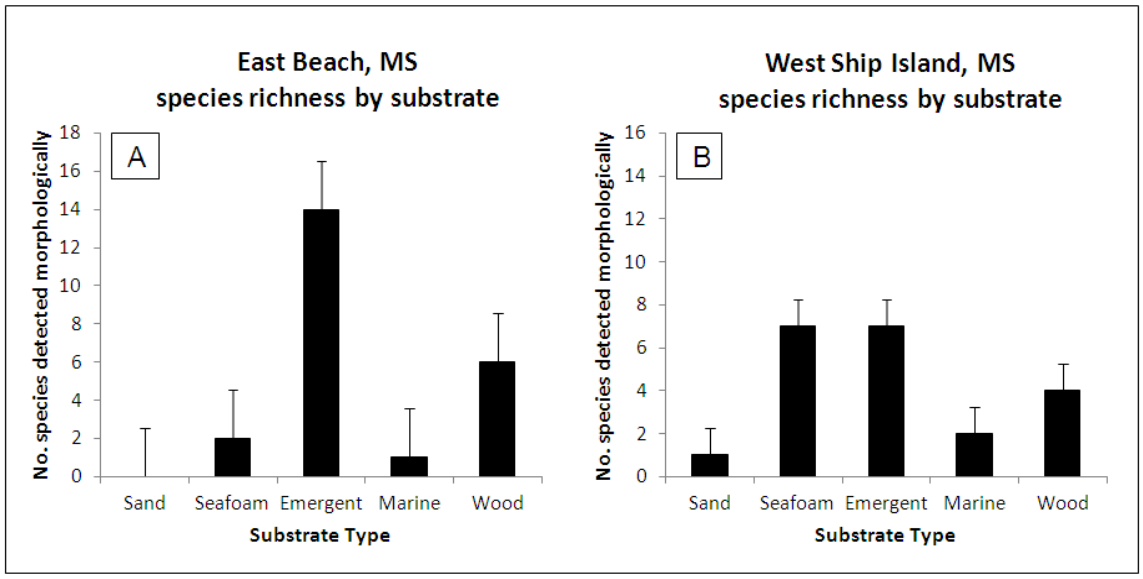

Figure 18. Marine ascomycete species richness by substrate at A) East Beach, Mississippi and B) West Ship Island, Mississippi. Error bars denote standard error.

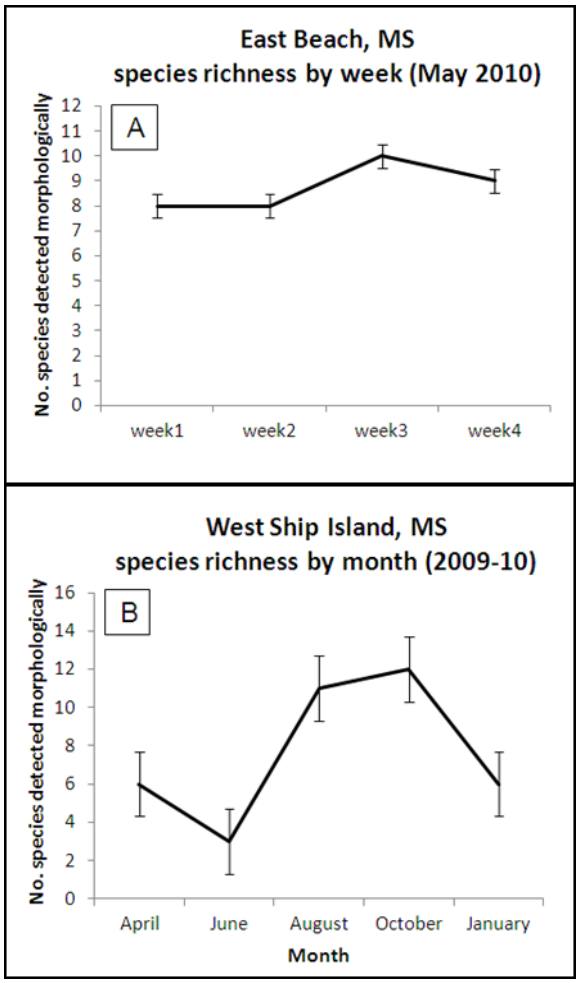

Figure 19. Marine ascomycete species richness by A) week, at East Beach, MS and B) month, at West Ship Island, MS. Error bars denote standard error.

Table 9

Detrital substrates collected at A) East Beach, MS in May 2010 by week, and B) West Ship Island, MS over ten months (April 2009-January 2010)

	Sand	Seafoam	Salt marsh	Algae	Seagrass	Wood
A)						
Week 1	x	x	x	x		x
Week 2	x	x	x			x
Week 3	x	x	x	x		x
Week 4	x	x	x			x
B)						
April	x	x	x	x		x

Table 9 (continued).

June	x	x			x	x
Aug	x	x	x	x		x
Oct	x	x	x	x	x	x
Jan	x	x	x	x	x	x

For emergent detritus (salt marsh) at East Beach, total species richness was similar each week, but the identity of the species collected changed from week to week during May 2010 (Table 10).

Table 10

Intertidal ascomycetes collected weekly on salt marsh detritus at East Beach, Mississippi in May 2010

Species	Week 1	Week 2	Week 3	Week 4
Sexual ascomycetes				
Anthostomella poecila	x	x	x	x
Atrotorquata lineata			x	
Buergenerula spartinae	x	x	x	x
Loratospora aestuarii				x
Massarina ricifera		x		
Massariosphaeria typhicola				x
Mycosphaerella sp. I	x	x		x
Mycosphaerella sp. II	x			
Passeriniella obiones	x	x	x	x
Phaeosphaeria halima	x	x	x	x
Phaeosphaeria roemeriani		x		x
Phaeosphaeria spartinicola	x	x	x	x
Pleospora pelvetiae			x	
Torpedospora radiata				x
Anamorphic ascomycetes				
Periconia sp.			x	
Tubercularia pulverulenta		x		

Sixteen marine ascomycete species were obtained from salt marsh plant detritus during weekly sampling at East Beach, MS during May 2010 (Table 9). Five species

were collected during all four weeks of sampling: *Phaesphaeria halima* and *Phaeosphaeria spartinicola* on *Spartina alterniflora* detritus, *Anthostomella poecila* on *Juncus roemerianus* detritus and *Passeriniella obiones* on unidentified *Spartina* sp. detritus. Species turnover was high, with the additional 11 species present during three of the sampling weeks or less. Eight of the species collected were only found during one of the four collection weeks.

Eight ascomycete species were isolated from salt marsh detritus during sampling events over ten months at West Ship Island, MS (Table 11). Seven species were isolated from seafoam, two were isolated from detrital wood, two from marine plant detritus and one species was isolated from sand.

Table 11

Intertidal ascomycetes collected at West Ship Island, Mississippi over ten months (April 2009 – January 2010). No salt marsh detritus was observed within the transect at the June 2009 sampling date

Species	April	June	August	October	January
Sand					
Corollospora maritima	x				x
Seafoam					
Acremonium alternatum		x			
Alternaria tenuissima				x	
Cladosporium sp.			x		
Cochliobolus hawaiiensis		x	x		
Corollospora maritima				x	x
Corollospora sp. 2					x
Paecilomyces variotii			x		
Marine plant detritus					
Corollospora maritima	x			x	
Varicosporina ramulosa	x	x		x	x
Saltmarsh detritus					
Anthostomella poecila			x	x	

Table 11 (continued).

Buergenerula spartinae		x	x		
Massarina ricifera		x	x	x	
Mycosphaerella sp. 1		x	x		
Passeriniella obiones	x	x	x	x	
Phaeosphaeria halima	x	x	x		
Phaeosphaeria spartinicola	x	x	x	x	
Phaeosphaeria olivacea	x				
Wood					
Trichocladium achrasporum			x		
Varicosporina ramulosa		x			

Discussion

Sampling weekly during the month of May may yield different results from samples obtained during other months of the year. Ideally, weekly sampling would be conducted during as many months as possible. Kohlmeyer et al. (2004) recommend sampling subtropical zones (which they define mycologically to include the mid-Atlantic U.S.) in winter and summer to fully capture the range of both temperate and tropical species expected.

The observed peak in species richness (S=12) for West Ship Island during October 2009 may have been due to the high substrate availability during that sampling event. October was the only sampling month in which all three solid substrate types were present in the target amounts within the 1 km transect (10 wood samples, 5 emergent plant detrital samples, 5 marine plant detrital samples). All substrate types were present in January, but in lesser quantities (5 wood samples, 4 emergent plant samples, 3 marine plant samples). More ascomycete species were isolated from seafoam at West Ship Island than at East Beach, which could be due to the higher wave energy at West Ship Island. Additionally, the samples to investigate intertidal fungal communities from East Beach,

MS taken during May 2010 may yield different results from other years due to the Deepwater Horizon oil spill (April 20, 2010).

The results of the current study suggest even more frequent sampling is required, with 14 species encountered in only one collection. Salt marsh detritus had 9 species each encountered in only one collection, indicating the need for more frequent sampling (at least weekly) to fully characterize the diverse marine ascomycete assemblage of this substrate. Higher sampling frequency is required for high lignocellulose-content substrates (wood, emergent plant detritus), due to their diverse fungal communities. Weekly collection of as many pieces of substrate as is feasible to be examined immediately is recommended. Species turnover was high on saltmarsh detritus between weeks at East Beach in May 2010. The number of marine fungal species identified morphologically from East Beach during this study was higher than from any FL or TX site, indicating the importance of sampling frequency in obtaining species richness estimates. Azevedo et al. (2012) found that 70 samples of drift substrates (emergent plant detritus, wood) were required to adequately assess marine ascomycete species richness for each substrate type on four sandy beaches in western Portugal.

Differences in substrate chemical composition and state of decay may account for differences observed in fungal assemblages between substrate types. Ascomycete species such as *Passeriniella obiones* are generalists, found on several substrate types containing high lignocellulose contents (intertidal wood and salt marsh detritus). *Corollospora maritima* is another example of a generalist species, found on sand, seafoam, wood and marine plant detritus. Most other species are substrate specific. Figueria and Barata

(2007) and Azevedo et al. (2012) also demonstrated intertidal fungal assemblages differ with substrate type, on sand beaches of western Portugal.

Tides and extreme weather events will strongly influence beach detritus availability. Sand beaches represent nearly 80% of ice-free coasts globally and yet ecological impact studies of extreme events such as hurricanes on these ecosystems are few (Dugan et al. 2010). In regions such as the GOM where tropical storms and hurricanes occur each year, efforts should be made to investigate the resultant changes in substrate availability on intertidal decomposer communities.

The recommended sampling protocol for seafoam is as follows: pooled sampling along a 1 km transect may suffice due to the low number of spores recovered in seafoam at all sites and in all seasons during this dissertation research. Allow spores to settle to bottom of collection bottle (1-2 hrs). Plate 1 mL full strength seafoam from bottom of bottle onto AWSA and spread with sterile spreader. Subculture fungal colonies to PDA daily for morphological and molecular identification. As well, directly observe seafoam under the microscope for the presence of ascospores, using lactophenol cotton blue stain, which stains chitin in fungal cell walls (and also crustaceans).

For sand, it is recommended to pool sand samples from multiple subsites along the 1 km transect, as low organic matter content and low species richness (S = 0-1) in sand was noted at all sites sampled. Few fungal reproductive structures were noted on sand in the absence of detrital substrates, suggesting that each piece of incoming detritus may serve as a microhabitat for its saprotrophic fungal community, bringing in fungi that do not establish on the sand beach outside of these microhabitats. However, spores of the fungal species present in intertidal detritus may be recovered from nearby seafoam.

Conclusions

When investigating intertidal fungal species richness, sampling effort, study design and substrate availability can have a large effect on the conclusions drawn. In the U.S. GOM, proximity to salt marsh and seagrass habitats, wind, storm events, tidal cycle and human beach impacts (beach renourishment, vehicular traffic) can influence substrate availability and hence intertidal fungal species richness at sand beach sampling sites. Weekly sampling on falling tides with direct observation of fungal reproductive structures is recommended, with a focus on high lignocellulose-content substrates from beaches having low human impact, to fully characterize sand beach fungal assemblages.

CHAPTER V

SUMMARY AND SIGNIFICANCE OF RESEARCH

This first inventory of marine ascomycetes of the U.S. GOM has increased the number of ascomycete species reported from the GOM by over 60% and forms the foundation of a comprehensive knowledge of the biodiversity and distribution patterns of marine ascomycetes in the U.S. GOM. Texas and Florida collections were completed prior to the Deepwater Horizon oil spill of 2010, providing baseline data on the marine mycota of the U.S. GOM. This dissertation includes an annotated checklist of the higher marine fungi of the entire GOM, a sampling protocol comprised of best practices for future collection of these organisms, as well as a collection of marine fungal dried specimens and cultures preserved and vouchered for future research.

1) Biodiversity. Substrate played the largest role in structuring intertidal fungal communities in this study, due in part to structural characteristics of the detritus and detrital availability. Emergent plant detritus and wood showed the highest fungal diversity of the substrates collected using both morphological and molecular techniques. The quantity and temporal availability of intertidal substrates can vary strongly as barrier island beaches are highly dynamic environments receiving differing amounts of flotsam on a daily basis. Wind, storm events and ocean circulation patterns likely play large roles in determining substrate availability for the intertidal saprotrophic ascomycete communities of GOM barrier island beaches.

Intertidal marine ascomycete diversity was found to increase with decreasing latitude in the U.S. GOM for certain substrates such as detrital seagrass. Seasonal differences in ascomycete communities were observed for marine plant detritus, which

exhibited higher diversity in winter than in summer. Reduced grazing pressure in winter may play a role. Molecular community fingerprinting via ITS T-RFLP analysis revealed the presence of 72% more ascomycete species for this region than morphological detection alone, revealing many ascomycete taxa present in the intertidal zone that were not fruiting at the time of collection.

A small number of dominant taxa of marine ascomycetes occur U.S. GOM-wide (*Corollospora maritima*, *Varicosporium ramulosa*). As many as 30% of the fungi encountered during this inventory were not identifiable due to lack of identification features, lack of material, or inability to culture and thus require further characterization.

2) Sucession. During laboratory incubation, sexual ascomycetes dominated substrate decay from 0-3 months. After 3-12 months, a community of asexual fungi and bacteria was noted. During the initial phase of decay when sexual ascomycetes dominated, no succession of ascomycete species was observed. This is in agreement with published evidence of fungi degrading higher molecular weight compounds into smaller compounds, which can then be utilized by other microorganisms.

3) Sampling Strategy. Intertidal substrates high in lignocellulose, such as wood, salt marsh and mangrove detritus, should be targeted for weekly collection in future studies of coastal marine fungi, as they exhibit the highest fungal species richness. Seafoam can provide a snapshot of spores present, but cannot indicate ecological role, unlike the presence of ascomata (fruiting bodies) on intertidal substrates. Pooled samples may suffice for seafoam and sand collection. Long-term laboratory incubation of detrital substrates may not be necessary as most sexual ascomycetes visible at the time of collection were also visible after 3-12 months laboratory incubation. Of the identifiable

fungi, many were present in only one collection. As the 4-week study at East Beach, MS indicated, more frequent sampling at each site is required to fully characterize these highly dynamic intertidal habitats. Weekly sampling of high lignocellulose-content substrates with immediate examination is recommended.

Future Directions

Metagenomics approaches and next generation gene sequencing will provide useful data for future studies of marine fungal diversity, and are beginning to be employed in this context (Amend et al. 2011). Denaturing gradient gel electrophoresis (DGGE) is another molecular technique for rapid assessment of fungal diversity that is only beginning to be employed in marine mycological studies (Gao et al. 2008). However, morphological identifications should also be done where possible, to fully elucidate the biology and adaptations of fungi capable of growing and reproducing in marine environments and to fully characterize these enigmatic communities and their potential as sources of novel compounds and genetic diversity.

APPENDIX A

PHYSICAL-CHEMICAL DATA FOR EACH COLLECTION SITE

Site	Location	Collection Season	GPS Coordinates N	W	Water temperature (C)	Salinity	pH
1	South Padre Island, TX	Winter 08-09	26 14 52.0	97 11 05.1	21.3	31.0	8.26
		Summer2009	26 14 52.0	97 11 05.1	26.6	31.2	8.35
2	Mustang Island State Park, TX	Winter 08-09	27 40 14.8	97 10 12.3	17.0	31.4	8.22
		Summer2009	27 40 14.8	97 10 12.3	28.5	30.1	8.27
3	Galveston Island State Park, TX	Winter 08-09	29 11 20.9	94 57 32.1	19.0	24.0	8.44
		Summer2009	29 11 20.9	94 57 32.1	31.1	29.5	8.28
4	West Ship Island, MS	April 2009	30 12 27.52	88 57 49.24	20.5	21.2	8.20
		June 2009	30 12 27.52	88 57 49.24	34.5	24.0	8.34
		August 2009	30 12 27.52	88 57 49.24	30.2	23.3	8.56
		October 2009	30 12 27.52	88 57 49.24	19.4	19.0	8.47

Site	Location	Collection Season	GPS Coordinates N	W	Water temperature (C)	Salinity	pH
4	West Ship Island, MS	May 2010	30 12 27.52	88 57 49.24	27.4	19.5	9.09
5	St. Vincent NWR, FL	Winter 08-09	29 40 36.0	85 13 15.3	17.6	24.9	8.34
		Summer2009	29 40 36.0	85 13 15.3	30.7	26.6	8.86
6	Caladesi Island State Park, FL	Winter 08-09	28 02 03.8	82 49 19.4	15.4	19.5	8.23
		Summer2009	28 02 03.8	82 49 19.4	30.2	35.0	7.80
7	Cayo Costa State Park, FL	Winter 08-09	26 41 17.9	82 15 29.5	16.0	31.6	8.08
		Summer2009	26 41 17.9	82 15 29.5	29.9	26.6	8.80
8	Bahia Honda State Park, FL	Winter 08-09	24 39 20.5	81 16 47.8	19.0	33.5	8.28
		Summer2009	24 39 20.5	81 16 47.8	29.8	31.1	8.60

APPENDIX B

CHECKLIST OF FUNGI FROM MARINE ENVIRONMENTS OF THE ENTIRE GULF OF MEXICO

List compiled from literature and this study (new records for the Gulf of Mexico recorded during this study/Walker and Campbell (2010) are indicated with an asterisk(*)). Habitat/biology codes: b = beach and shoreline, itd = intertidal, m = mangrove, sm = saltmarsh, sg = seagrass, f = seafoam, sft = soft substrates, w = detrital wood, par = parasitic, end = endemic to GOM. Updated from González (2009).

Taxon	Habitat/Biology	Known Range	GOM Range	References
Phylum: Ascomycota				
Class: Dothideomycetes				
Order: Capnodiales				
Family: *Mycosphaerellaceae*				
Mycosphaerella sp. I*	itd, sm	Atl	NC	Walker & Camp. 2010
Mycosphaerella sp. II*	itd, sm	Atl	NC	Walker & Camp. 2010
*Sphaerulina orae-maris** Linder 1944	itd, w	Atl, Pac	NC	Linder 1944
Order: Dothideales				
Family: *Planistromellaceae*				
*Loratospora aestuarii** Kohlm. & Volkm.-Kohlm. 1993	itd	Atl	NC	Kohlm. & Volkm.-Kohlm. 1993
Family: *Incertae sedis*				
Lineolata rhizophorae Kohlm. & Volkm.-Kohlm. 1990	itd, m, sm	Atl, Med, Pac	NE	Koehn 1985
Paraliomyces lentiferus Kohlm. 1959	itd, m	Ind	NE, SW	Kohlm. & V-K. 1979
Order: Pleosporales				
Family: *Didymosphaeriaceae*				
Verruculina enalia Kohlm. & Volkm-Kohlm. 1990	itd, m, b	Atl, Pac	NE, SW	Kohlm. & Volkm-Kohlm. 1990b

Taxon	Habitat/Biology	Known Range	GOM Range	References
Family: *Massarinaceae*				
*Massarina ricifera** Kohlm., Volkm.-Kohlm. & O.E. Erikss. 1995	itd, sm	Atl	NC	Kohlm. et al. 1995
Family: *Phaeosphaeriaceae*				
*Phaeosphaeria halima**(T.W. Johnson) Shoemaker & C.E. Babc. 1989	itd, sm	Atl	NC	Shoemaker & Bab. 1989
*Phaeosphaeria olivacea** Kohlm., Volkm.-Kohlm. & O.E. Erikss. 1997	itd, sm	Atl	NC	Kohlm. et al. 1997
*Phaeosphaeria roemeriani** Kohlm., Volkm.-Kohlm. & O.E. Erikss. 1998	itd, sm	Atl	NC	Kohlm. et al. 1998
*Phaeosphaeria spartinicola** Leuchtm.	itd, sm	Atl	NC	Leuchtm. & New. 1999
Family: *Leptosphaeriaceae*				
Leptosphaeria australiensis G.C. Hughes 1969	itd	Atl, Ind, Pac	NE	Hughes 1969
Leptosphaeria avicenniae Kohlm. & E. Kohlm. 1965	itd, m	Atl	NE, SE*, SW	Kohlm. & Kohlm. 1965
*Leptosphaeria contecta** Kohlm. 1963	itd, sm	Atl, Pac	NC	Walker & Campb. 2010
Leptosphaeria marina Everhart 1885	itd, sm	Atl	NW, NE	Johnson 1956
Leptosphaeria oraemaris Linder 1944	itd, sm	Atl, Med, Pac	NW	Johnson 1956
Leptosphaeria pelagica E.B.G. Jones 1962	itd, sm	Atl, Pac	NW	Jones 1962
Family: *Lophiostomataceae*				
Etheirophora blepharospora Kohlm. & Volkmann-Kohlm. 1989	m	Atl, Pac	SW, NE	Kohlm. & V.-K. 1989
Family: *Melanommataceae*				
Trematosphaeria mangrovis Kohlm. 1968	m	Atl	NE	Kohlm. 1968
*Acrocordiopsis patilii** Borse & K.D. Hyde 1989	itd, m	Ind	SE	Borse & Hyde 1989

Taxon	Habitat/Biology	Known Range	GOM Range	References
Family: *Pleosporaceae*				
*Pleospora pelvetiae** G.K. Sutherl. 1915	itd, sm	Atl, Pac	NC	Yang et al. 2009
Family: *incertae sedis*				
*Massariosphaeria typhicola** (P. Karst.) Leuchtm. 1984	itd, sm	Atl, Pac	NC	Leuchtmann 1984
Order: incertae sedis				
Family: *incertae sedis*				
*Passeriniella obiones** (P. Crouan & H. Crouan) K.D. Hyde & Mouzouras 1988	itd, sm, w	Atl	NC	Hyde & Mouzouras 1988
Class: Sordariomycetes				
Order: Diaporthales				
Family: *Valsaceae*				
Cytospora rhizophorae Kohlm. & Kohlm. 1971	m	Atl, Pac	SW	Kohlm. & K. 1977
Order: Incertae sedis				
Family: *Incertae sedis*				
Torpedospora radiata Meyers 1957	itd, sm	Pac	SW, SE, NC*	Kohlm. 1968
Order: Incertae sedis				
Family: *Magnaporthaceae*				
*Buergenerula spartinae** Kohlm. & R.V. Gessner	itd, sm	Atl	NW, NC	Walker & Campb. 2010
Order: Xylariales				
Family: *Cainiaceae*				
*Atrotorquata lineata** Kohlm.& VK 1993	itd, sm	Atl	NC	Kohlm. & V.-K. 1993
Family: *Xylariaceae*				
*Anthostomella poecila** Kohlm., Volkm.-Kohlm. & O.E. Erikss. 1995	itd, sm	Atl	NC	Kohlm. et al. 1995

Taxon	Habitat/Biology	Known Range	GOM Range	References
Order: Halosphaeriales				
Family:*Halosphaeriaceae*				
Antennospora quadricornuta Johnson 1958	b, itd, sft	Atl, Ind, Pac	NW, NE	Johnson 1958
Antennospora salina Johnson 1958	b, itd, sft	Atl, Pac	SW	Johnson 1958
Arenariomyces parvulus Koch 1986	b, itd, sft	Atl, Pac	SW, SE	Koch 1986
Arenariomyces triseptatus Kohlm. 1984	b, itd, sft	Atl	SE	Kohlm. 1984
Ceriosporopsis halima Linder 1944	b, itd, sft	Atl, Med, Pac	SW, SE	Linder 1944
Corollospora angusta Nakagiri & Tokura 1987	b, itd, sft	Pac	SW	Nakagiri & Tokura 1987
Corollospora armoricana Kohlm. & Volkm.-Kohlm. 1989	b, itd, sft	Atl	SE	Kohlm. & V-K 1989
Corollospora cinnamomea Koch 1986	b, itd, sft	Ind	SE	Koch 1986
Corollospora gracilis Nakagiri & Tokura 1987	b, itd, sft	Pac	SW	Nakagiri & T. 1987
*Corollospora intermedia** Jones 1970	itd, *Sargassum*	Atl	SW	Jones 1970
Corollospora lacera Kohlm. 1962	b, itd, sft	Atl, Pac	NE	Kohlm. 1970
Corollospora maritima Werderm. 1922	b, itd, sft	Atl, Med, Pac	Entire	Wagner-Merner 1972
Corollospora pseudopulchella Nakagiri & Tokura 1987	b, itd, sft	Atl, Pac	SE	Nakagiri & Tokura 1987
Corollospora pulchella Kohlm, Schmidt & Nair 1967	b, itd, sft, f	Atl, Ind, Pac	NW	Kohlm. et al. 1967
Corollospora quinqueseptata Nakagiri 1987	b, itd, sft	Pac	SE	Nakagiri 1987
Corollospora trifurcata Höhnk (Kohlm.) 1962	b, itd, sft	Atl, Ind, Med, Pac	NE, SE	Kohlm. & Kohlm. 1979
Corollospora sp. 1*	itd, foam	-	NC	-
Corollospora sp. 2*	itd, *Sargassum*	-	SW	-
Corollospora sp. 3*	itd, sg, green alga	-	SE	-
Crinigera maritima Schmidt 1985	m	Atl, Pac	NE	Schmidt 1985
Falcatispora cincinnatula (Shearer & J.L. Crane) K.L. Pang & E.B.G. Jones 2003	itd	Atl, Ind	SE	Pang et al. 2003

Taxon	Habitat/Biology	Known Range	GOM Range	References
Halosphaeriopsis mediosetigera Cribb & J.W. Cribb) T.W. Johnson 1958	itd	Atl, Pac	SW	Johnson 1958
*Haiyanga salina** (Meyers) K.L. Pang & E.B.G. Jones 2008	itd, m	Atl, Ind, Pac	SE	Pang et al. 2008a
Lignincola laevis Höhnk 1955	m	Atl, Ind, Pac	SE	Kohlm. & Kohlm. 1979
Lignincola tropica Kohlm. 1984	b	Ind, Pac	SE	Kohlm. 1984
Order: Hypocreales				
Family:*Bionectriaceae*				
Kallichroma tethys Kohlm. & Volkmann-Kohlm. 1993	m	Atl, Ind, Pac	NE	Kohlm. & Volkmann-Kohlm. 1993
Order: Lulworthiales				
Family:*Lulworthiaceae*				
Lindra inflata Wilson 1956	itd	Atl	NW	Wilson 1956
Lindra thalassiae Orpurt, Meyers, Boral & Simms 1964	itd, sg	Atl, Pac	SW, NC, NE, SE	Orpurt et al. 1964
Lindra sp.1*	itd, f	-	SW	-
Lindra sp. 2*	itd, w	-	NC	-
Lulworthia grandispora Meyers 1957	b, m	Atl, Ind, Pac	SE	Meyers 1957
Lulworthia fucicola Sutherland 1916	b, itd	Atl, Pac	SE	Sutherland 1916
Lulworthia kniepii Kohlm. 1963	par	Atl, Med, Pac	NW, SE	Kohlm. 1963
Lulworthia medusa Cribb & J.W. Cribb 1956	sm	Atl, Ind, Pac	NW	Cribb & Cribb 1956
Family:*Incertae sedis*				
*Haloguignardia decidua** Cribb & J.W. Cribb 1956	itd, f	Pac	NC*	Cribb & Cribb 1956
Order: Sordariales				
Family:*Incertae sedis*				
Savoryella lignincola E.B.G. Jones & Eaton 1969	b	Atl, Ind, Pac	SE	Eaton & Jones 1971

Taxon	Habitat/Biology	Known Range	GOM Range	References
Anamorphic Ascomycetes				
*Acremonium alternatum**	itd, f		NC	
*Alternaria tenuissima**	itd, f, w		NC, NW	
Cirrenalia basiminuta Rhaghuk&Zainal 1988	itd, m	Pac	SE	Raghukumar et al. 1988
Cirrenalia pseudomacrocephala Kohlm. 1968	m	Atl	SW	Kohlm. 1968
Cladosporium sp.* Link 1816	itd, f		NC, SE	
*Cochliobolus hawaiiensis**Alcorn 1978	itd, f		NC	Alcorn 1978
Halenospora varia (Anastasiou) E.B.G. Jones 2009	itd, b	Atl, Ind, Pac	NE, SE	Jones et al. 2009
Humicola allopallonella Meyers & Moore 1960	b, itd	Atl, Ind	SE	Meyers & Moore 1960
Lentescospora submarina Linder 1944	sm	Atl	NW	Linder 1944
*Paecilomyces variotii**	itd		NC	
Periconia sp.*	itd, sm		NC	
Pontogeneia cubensis Kohlm. 1975	b, end	GOM	SE	Kohlm. 1975
Scolecobasidium arenarium Ellis 1976	sg	Atl	NW	Ellis 1976
Trichocladium achrasporum Shearer & Crane 1971	itd, w	Atl, Ind, Pac	SW, NE, NC*	Shearer & Crane 1971
*Tubercularia pulverulenta** Speg. 1881	itd, sm	Atl	NC*	Kohlm. & Kohlm. 1979
Varicosporina ramulosa Meyers & Kohlm. 1965	itd	Atl, Pac	SW, SE, NE	Meyers & Kohlm. 1965
*Varicosporina prolifera** Nakagiri 1986	itd, *Sargassum*	Pac	SE*	Nakagiri 1986
*Zalerion maritima** (Linder) Anastasiou 1963	itd	Atl, Ind, Med, Pac	NC	Anastasiou 1963

Taxon	Habitat/Biology	Known Range	GOM Range	References
Phylum: Basidiomycota				
Class: Agaricomycetales				
Order: Polyporales				
Family: *Cyphellaceae*				
Halocyphina villosa Kohlm. & Kohlm. 1965	b, m	Atl, Ind, Pac	NE	Kohlm. 1980
Order: Agaricales				
Family: *Niaceae*				
Nia vibrissa R.T. Moore & Meyers 1959	b, itd	Atl, Med, Pac	NE	Kohlm. 1980
Phylum: Cercozoa				
Class: Phytomyxea				
Order: Plasmodiophorida				
Family: *Plasmodiophoraceae*				
Protist: *Plasmodiophora diplantherae** (Ferd. & Winge) Ivimey Cook 1933	par on *H. wrighti*	Atl, Ind, Pac	NC*, SE*	Walker & Campb. 2009

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